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GENOME MAPPING RESEARCH - PLANTS

A Directory of USDA and State Projects in CRIS

Prepared by

Current Research Information System Cooperative State Research Service

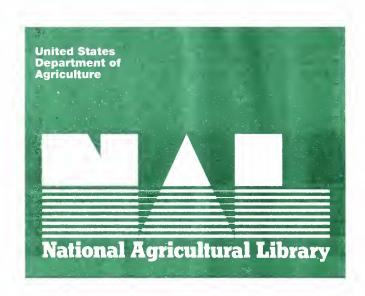
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January 1990

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CURRENT RESEARCH INFORMATION SYSTEM

The Current Research Information System (CRIS) is a computer-based information storage and retrieval system which documents and provides access to information on publicly supported agricultural and forestry research in the United States. CRIS is operated by the Cooperative State Research Service, U.S. Department of Agriculture (USDA).

The CRIS database consists of descriptions of research projects conducted or sponsored by the USDA research agencies, the State agricultural experiment stations and land-grant institutions, State forestry schools, Tuskegee University, U.S. schools of veterinary medicine, and participants in the competitive and special research grants program of the Department.

Approximately 30,000 ongoing and recently completed research projects are documented in CRIS. Some 5,000 new projects and revisions are entered in the system annually. Most projects remain active for 3-5 years and are retained in the file an additional 3-4 years beyond their termination date. Active projects are updated annually with progress reports and citations to the latest publications resulting from the research.

Retrieval of CRIS project information is provided to users worldwide through the publicly accessible CRIS/USDA online file (File 60) on DIALOG, the online retrieval system of DIALOG Information Services, Inc.

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PREFACE

This directory is a selected listing of research projects dealing with genome mapping in major crop commodities. The source for all projects was the Current Research Information System (CRIS), the research project documentation and reporting system of the U.S. Department of Agriculture (USDA).

The CRIS system contains projects conducted or sponsored by the USDA research agencies, the State agricultural experiment stations and land-grant institutions, U.S. schools and colleges of veterinary medicine, and other cooperating State institutions. Subject coverage includes the area of agriculture and forestry, in all its broad aspects, as well as food and human nutrition, environmental quality, and rural development. CRIS is operated by the Cooperative State Research Service, USDA.

Projects in this directory were retrieved from the base of projects in CRIS as of September 22, 1989. From the total of 1,845 projects initially retrieved on genome mapping, 860 were judged to have 50% or greater relevance to the topic. Distribution within the 860 projects for the plant, animal and other commodity groupings is as follows:

Commodity	No. of Projects*
Plants Animals Other	427 255 223

*includes overlap

Individual research directories were prepared based on this distribution, with titles reflecting the particular content of each directory.

Full project abstracts appear in this directory in the main entry section entitled, "Research Project Descriptions." The section is divided into chapters based on commodity classifications used for classifying projects in the CRIS system. Abstracts appear in chapters that correspond to the commodity classifications assigned when the projects were submitted for entry in CRIS. Projects assigned more than one commodity classification are repeated in each of the applicable chapters. Repeat entries are identified by an asterisk next to the directory number in the main entry section and in the indexes.

Arrangement of projects within chapters is alphabetical by State or country, followed in order by name of performing institution, department, and principal investigator.

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The three indexes provided are the Keyword/Title Index, Investigator Index, and Performing Institution Index. Directory numbers in the indexes are used for locating projects in the main entry section. The two left-most digits correspond to the chapter in which the project is located, and the three right-most digits indicate its position within the chapter.

Index terms in the Keyword/Title Index are the keywords originally assigned by CRIS primarily for use in computer retrieval. Project titles have been inserted to provide context.

The Investigator Index is an alphabetical listing of the principal and co-investigators on the projects.

The Performing Institution Index lists the names of the institutions alphabetically by city within the State or country.

To obtain abstracts of newer projects or for later updates of progress and publications on projects listed here, users may directly access the CRIS online file, CRIS/USDA (File 60), on DIALOG, the online retrieval system of DIALOG Information Services, Inc. The CRIS accession number shown opposite the directory number in the main entry section may be used with a TYPE or DISPLAY command on DIALOG to retrieve project information online at the user's terminal. Format 7 in the online file carries the narrative summary including the most recent update of progress and publication citations entered on the project. Projects remain on the file for 3-4 years beyond their termination date and are then purged. The file is updated monthly.

This directory was compiled by Philip L. Dopkowski and F. Allen Moore, Technical Products and Services, CRIS. Edward A. Warnick, Information Systems Division, National Agricultural Library, provided technical assistance. Dr. Bruno Quebedeaux, Cooperative State Research Service, provided scientific oversight and support in the review and assessment of projects for relevancy.

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RESEARCH PROJECT DESCRIPTIONS

CM 06 TREES, FORESTS, AND FOREST PRODUCTS

O6.001 CRISO133181
MECHANISMS OF INHERITANCE AND TRANSMISSION OF
CONIFER ORGANELLE GENOMES

NEALE D B; SEDEROFF R R; Forest Genetics; 1960 Addison Street, Berkeley, CALIFORNIA 94704. Proj. No.: CALR-8701957 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 87 to 31 AUG 90

Objectives: PROJ. 8701957. Determine the mode of chloroplast inheritance in conifers. Determine the mode of mitochondrial inheritance in conifers. Determine the stages of development when organelle exclusion occurs. Determine the frequency of maternal or nonparental transmission of chloroplast DNA. Determine if heteroplasmy exists in the chloroplast DNA of Douglas-fir. Determine if Douglas-fir cpDNAs are rearranged during transmission through pollen. Determine by DNA sequence analysis the mechanisms of rearrangement in Douglas-fir cpDNA.

Approach: Organelle inheritance will be studied by identifying RFLPs in parent trees of full-sib crosses. Progeny will then be assayed to infer inheritance. RFLP analysis involves DNA isolation, Southern transfers, and DNA hybridizations with heterologous probes. Nechanisms of cpDNA rearrangement will be studied by isolation and plasmid cloning of variable DNA fragments and DNA sequencing of M13 clones by the chain termination procedure.

Progress: 88/01 to 88/12. We have been investigating the inheritance and transmission of conifer organelle genomes. We have completed the study of chloroplast DNA inheritance and have shown that cpDNA is paternally inherited in Douglas-fir, loblolly pine, incense-cedar. and coast redwood. Based on the results from these taxa and other published reports, it appears that cpDNA is strictly paternally inherited in conifers. We have shown tht mtDNA is maternally inherited in loblolly pine. however preliminary evidence suggests that mtDNA is paternally inherited in coast redwood and incense-cedar. We have not been able to detect any evidence for heteroplasmy in chloroplast genomes of conifers. We genotyped 50 offspring from each of three Douglas-fir crosses and all had the paternal genotype. In addition we found no differences among samples from individual trees and among pollen samples collected from these trees. We suggest that heteroplasmy is extremely rare in Douglas-fir cpDNA. We have begun the sequence analysis of the rearranged cpDNA fragments in Douglas-fir and will complete this during the next year.

Publications: 88/01 to 88/12 NEALE, D.B. and SEDEROFF, R.R. 1988. Inheritance and evolution of conifer organelle genomes. In "Genetic Manipulation of Woody Plants," J. Hanover and D. Keathley (eds.), Plenum Press, New York. NEALE, D.B. and SEDEROFF, R.R. 1988. Paternal inheritance of chloroplast DNA and maternal inheritance of nitochondrial DNA in loblolly pine. Theor. Appl. Genet. (in press). NEALE, D.B., MARSHALL, K.A. and SEDEROFF, R.R. 1988. Inheritance of chloroplast and mitochondrial DNA in conifers. Studia Forestalia Suecica (in press). STRAUSS, S.H., NEALE, D.B. and WAGNER, D.B. 1989. Genetics of the chloroplast in conifers: Biotechnology research reveals

O6.002 CRISO136418 CONSTRUCTION OF A SATURATED GENETIC LINKAGE MAP FOR LOBLOLLY PINE

some surprises. J. Forestry (in press).

NEALE D B; KINLAW C S; Pacific Southwest Forest and Range Experiment Station; 1960 Addison Street, Berkeley, **CALIFORNIA** 94704. Proj. No.: CALR-8802733 Project Type: CRGO Agency ID: CRGO Period: 30 SEP 88 to 30 JUN 91

Objectives: PROJ. 8802733. Characterize the organization of the loblolly pine genome by mapping 300 random cDNA clones. Characterize the distribution of functionally related genes and members of gene families by mapping some specific classes of genes.

Approach: Identify 1-2 crosses made among the most highly heterozygous parent trees based on isozyme analysis. Pedigrees will be provided by the North Caroline State University Tree Improvement Cooperative. Construct a loblolly pine cDNA library in the phagemid vector pCDM8. Isolate DNA from parent trees and their progeny, prepare Southern blots, and identify 300 polymorphic loci based on RFLPs. Identify RFLPs in crosses for several genes in each of three gene classes. Construct the genetic map using the Lander and Green (1987) algorithm and the computer program, MAPMAKER.

O6.003 CRISO130376
GENETIC MOSAICS AND THEIR RELATIONSHIP TO
PATTERNS OF SUSCEPTIBILITY TO INSECTS AND
DISEASES

HDUSTDN D R; HOUSTDN D B; USDA Forest Service; 51 Mill Pond Road, Hamden, CONNECTICUT 06514.
Proj. No.: CDNR-RWU-4505 Project Type: CRGD Agency ID: CRGD Period: 30 SEP 86 to 29 SEP 88

Objectives: PRDJECT 8602560. Determine the patterns of occurrence (levels of occurrence and spatial distribution) of trees resistant to beech bark disease in long-affected forests. Determine the genetic relationship of trees resistant to beech bark disease. Determine if distribution patterns and genetic relationships (as reflected by isozyme genotypes) are representative of the general beech population.

Approach: Resistant beech trees in 10 forest stands in Maine and Canada will be located and mapped to obtain information on their levels and spatial distribution patterns. Genetic relationships of all resistant trees in 4 stands will be determined by comparing isozyme profiles. Age-growth data will be analyzed to determine dates of establishment or release of trees in and among groups. Patterns revealed by analysis of resistant trees will be compared to those for the general beech population.

Progress: 87/10 to 88/09. This study entails the analysis of the age/growth structure and isozyme genetics of beech (Fagus grandifolia) trees resistant and susceptible to the beech scale (Cryptococcus fagisuga) in relation to their spatial distribution patterns. In FY 1988, all the resistant trees in 4, 25 ac forest stands in Canada were located and mapped, and in 2 stands increment cores were obtained. Buds from all resistant trees in 2 of these stands, and from resistant trees in 2 similar stands in Maine (see 1987 report) were harvested for isozyme analyses. In 1 of the Maine stands, all susceptible trees in a .75 ac area were mapped and cores were collected. All cores from resistant trees were read. Yearly growth increments and tree ages have been stored for future analyses. Isozymes were extracted and isozyme genotypes determined (based on 9 polymorphic isozymes) for all the resistant trees (203) in the Maine plots. This work will continue in FY 1989.

Publications: 87/10 to 88/09
HOUSTON, D.R. and HDUSTDN, D.B. 1987.
Resistance in American beech to
Cryptococcus fagisuga: Preliminary findings
and their implications for forest
management. p. 105-116 In Proc., 30th
Northeastern forest tree improvement conf.

O6.004 CRISO131668
INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF
CHLOROPLAST DNA POLYMORPHISMS IN LODGEPOLE AND
JACK PINES

WAGNER D B; Forestry; University of Kentucky, Lexington, **KENTUCKY** 40506. Proj. No.: KY00640

Project Type: MCINTIRE-STENNIS

Agency ID: CSRS Period: 01 JUL 87 to 30 JUN 92

Objectives: Develop physical maps of chloroplast genomes of lodgepole and jack pines; Characterize the molecular basis of cpDNA restriction fragment length variants; Investigate the mode of inheritance of cpDNA in intra- and interspecific progenies; Determine effects of herbicide and drought stresses on cpDNA population dynamics, survival and growth.

Approach: Purification of chloroplast DNA of identified sample trees followed by development of restriction-site map of chloroplast genome of each species, hybridization of nick-translated radio-labelled heterologous genes, autoradiography; Using purified genomic cpDNA from as probes, examine cpDNA variants within species to infer base substitutions, inversions, deletions/insertions and calculate rate of evolution of coniferous cpDNA; Infer cpDNA genotypes from total cellular DNA of reciprocal interspecific crosses; Using open-pollinated families, determine allozyme genotypes and cpDNA genotypes from seedling tissue, expose seedlings to herbicide and drought treatments and determine differences in genotypic frequencies between resistant (surviving) and susceptible phenotypes.

Progress: 88/01 to 88/12. This project began July, 1987, and is still in the early stages of work. Substantial progress has been made toward understanding the molecular basis of chloroplast (cp) DNA polymorphism in Pinus banksiana and P. contorta during the past year. It is now clear that cpDNA variants are predominantly due to short insertions and deletions. Moreover, the most frequent insertions/deletions occur in a region that contains short repeated sequences and that is upstream from a gene duplication. This is consistent with mechanisms that have been hypothesized to generate cpDNA polymorphism in other plants. A cloned sequence, which we have been using to map cpDNA variation, fortuitously hybridizes with many restriction fragments from the P. contorta chloroplast genome, suggesting that a cpDNA sequence is repeated many times. The restriction fragments identified with this clone are variable in size among individuals, of P. banksiana and P. contorta; this variation may be useful in paternity analyses. We have found that the most polymorphic region of the P. banksiana - P. contorta chloroplast genome is monomorphic in other members of the genus Pinus. This inconsistency is consistent with results in conifers from other laboratories. Portions of this work were presented at one scientific conference in 1988, and one manuscript has been submitted for publication.

Publications: 88/01 to 88/12
STRAUSS, S.H., NEALE, D.B. and WAGNER, D.B.
 (1988). Genetics of the chloroplast in
 conifers: biotechnology research reveals
 some surprises. J. For. (In Press).

06.005 CRISO134775 REGULATION OF GENE EXPRESSION DURING DEVELOPMENT AND MATURATION IN CONIFERS

HUTCHISDN K W; Biochemistry; University of Maine, Orono, ${\bf MAINE}$ O4469. Proj. No.: MEO9401

Project Type: MCINTIRE-STENNIS
Agency ID: CSRS Period: O1 DCT 88 to 30 SEP 93

Objectives: The continued identification and characterization of genes that are differentially expressed in mature and juvenile meristemic tissue. The correlation of level of expression with genetic background. Determination of the level of expression of the small subunit of ribulose-1,5-bisphosphate and light harvesting complex protein genes in mature and juvenile foliage. The analysis of clones for low copy number sequences for the presence of restriction fragment length

Approach: This work will be approached using the techniques of recombinant DNA, to clone and identify sequences expressed in the meristem of juvenile and mature larch trees. DNA libraries will be characterized using differential and subtractive hybridization.

polymorphisms.

O6.006 CRISO133267 GENE EXPRESSION DURING MATURATION IN CONIFERS IN RELATION TO APPLICATION OF BIOTECHNOLOGY

GREENWDDD M; HUTCHISDN K; Forest Biology; University of Maine, Drono, MAINE 04469. Proj. No.: ME35170 Project Type: CRGD Agency ID: CRGD Period: 15 SEP 87 to 30 SEP 90

Objectives: The continued identification and characterization of genes that are differentially expressed in mature and juvenile meristemic tissue. The correlation of level of expression with genetic background.

Determination of the level of expression of the small subunit of ribulose-1,5-bisphosphate carboxylase (ssu-Rubisco) and light harvesting complex protein (LHCP) genes in mature and juvenile foliage. The analysis of clones for low copy number sequences for the presence of restriction fragment length polymorphisms.

Proj. 8701986.

Approach: Genes that are differentially expressed in juvenile and mature plants will be identified by reciprocal hybridization of cDNA libraries. Genetic variation will be determined by probing RNA from different tree families. The same RNA will be used to determine the level of expression of the ssu-Rubisco and LHCP genes. Restriction fragment length polymorphisms will be detected by genomic Southern blots of DNA from at least 10 tree families, digested with a variety of restriction enzymes.

Progress: 87/10 to 88/09. We have continued our collaborative study on the molecular genetics of maturation in conifers, and have shown that genes encoding the chlorophyll

a/b-binding proteins (Cab) are expressed in a maturation related manner. We extended the initial study to include a wide spectrum of ages of trees analyzed, ranging from 1-year-old trees to a 74-year-old tree. The data suggest that when the amount of expression of the Cab genes is compared to the expression of other genes, the bulk of the decrease in level of expression occurs in the first 5 years of age. This correlates well with the physiological and morphological data on maturation in larch, and suggests that there may be a common mechanism involved. In addition, we have initiated a study to look directly at gene expression in two maturation-related phenomenon, namely root formation, and cone formation. In the three years of this research project we have developed the technology necessary for the molecular genetic investigation of development in conifers. We found, upon initiation of this project that most of the current recombinant DNA technology was not directly transferrable to working with conifers. This was due to a combination of unique contaminating compounds in conifer tissues, and the complexity of the conifer genome. We have now adapted the techniques necessary to proceed with our studies on conifer development and maturation.

Publications: 87/10 to 88/09

HUTCHISDN, K.W., SINGER, P.B., and GREENWOOD, M.S. 1988. Molecular analysis of gene expression during the development and maturation of larch. In: W.M.

Cheliak and A.C. Yapa (eds), Molecular Genetics of Forest Trees.

HUTCHISDN, K.W., SINGER, P.B., and GREENWOOD, M.S. 1988. Molecular analysis of gene expression during the development and maturation of larch. In: J.W. Hanover and D.E. Keathley (eds). Genetic Manipulation of Woody Plants Symposium.

HUTCHISDN, K.W., SINGER, P.B., and GREENWDDD, M.S. 1988. Gene expression during growth and maturation of the conifer Larix laricina. J. Cell. Biochem. 12C:211.

O6.007 CRISO097665 GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE

HANDVER J W; KEATHLEY D E; Forestry; Michigan State University, East Lansing, **MICHIGAN** 48824.

Proj. No.: MICLO8029 Project Type: CRGD Agency ID: CRGD Period: 15 SEP 85 to 31 DEC 88

Objectives: Proj 8508203. Determine the breeding structure including interspecific hybridization of natural populations of blue spruce, Engelmann spruce, white spruce, and mixed populations of all species. Characterize simply inherited traits and determine the genetics of adaptive traits in tree species.

Approach: Conduct half- and full-sib progeny analyses of spruce species and hybrids for morphological, biochemical, and physiological traits. Determine species crossability and gene control of important traits.

Progress: 85/09 to 88/12. Earlier in the project we completed a partial diallel controlled mating design involving 20 parents of both species in the study area, including crosses both within and between the species. The species crossed with great difficulty and only in one direction with blue spruce as the pollen or male parent. We obtained the first documented viable hybrids between the two species which were verified by isozyme markers and cpDNA restriction fragment length polymorphisms. Chloroplast DNA (cpDNA) was purified from Picea glauca, P. pungens, P. engelmannii, and P. omorika, and was digested with several restriction endonucleases. Interspecific restriction fragment length polymorphisms (RFLPs) of cpDNA were identified. The RFLPs were identified as cpDNA by the hybridization of cloned, -P labeled, petunia cpDNA to the polymorphic bands, and by the lack of hybridization of a cloned and labeled mtDNA probe from maize. Chloroplast DNA RFLPs that showed no intraspecific variation when examined across the natural range for each species were used as markers to follow the inheritance of plastids in interspecific hybrids. The inheritance of plastids was determined for F(1)-hybrids from reciprocal crosses of P. glauca and P. pungens, P. glauca and P. omorika, and F(1)-hybrids of P. engelmanni x pungens. All 31 F(1)-hybrids examined showed the cpDNA genotypes of the pollen parent, or the paternal species.

Publications: 85/09 to 88/12

- ERNST, S. HANOVER, J.W., KEATHLEY, D.E. and MAO, I.L. 1988. Genetic variation and control of intraspecific crossability in blue and Engelmann spruce. Silvae Genetica 37:112-118.
- STINE, M. and KEATHLEY, D.E. 1988. Evidence for paternal inheritance of plastids in interspecific hybrids of Picea. In: Genetic Manipulation of Woody Plants, J.W. Hanover and D.E. Keathley, eds. Plenum Press, New York and London, p. 486.
- York and London. p. 486. SCHUTZKI, R.W. 1988. Effects of water stress on Picea seedlings. Ph.D. Thesis, Michigan State University. 176 pp.
- STINE, M. 1988. Demonstration of paternal inheritance of plastids in Picea (Pinaceae). Ph.D. Thesis, Michigan State University. 63 pp.

06.008 CRISO130225 DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS

KEATHLEY D E; Forestry; Michigan State University, East Lansing, **MICHIGAN** 48824. Proj. No.: MICLO1487

Project Type: MCINTIRE-STENNIS
Agency ID: CSRS Period: O1 OCT 86 to 30 SEP 91

Objectives: Develop efficient callus, bud, and protoplast culture systems for physiologically mature conifers and incorporate this technology into breeding programs. Develop callus and protoplast culture systems for promising hardwood species, and work on gene vector systems for these species. Use the culture systems to gain a better understanding genome

organization in woody plants, and to facilitate molecular genetics studies of these species.

Approach: The culture systems will be developed by starting with standard media and then altering the phytohormones, vitamins, and other media components until the desired form of growth is obtained. The molecular genetics studies will be conducted using restriction endonucleases to identify fragment polymorphisms (RFLPs) in different individuals and between species. These will then be used to start the process of mapping the organelle and nuclear genomes.

Progress: 88/01 to 88/12. Progress continued in developing tissue culture systems for both hardwood and conifer species. For Black locust, differential responses in vitro for different genotypes were demonstrated, and the growth of callus from protoplast cultures were obtained. Cell lines infected by Agrobacterium tumefaciens were analyzed and shown to contain T-DNA. Work on the development of tissue culture of Christmas tree species continued, with protocols that allow increased elongation of regenerated being elucidated. Studies on the molecular genetics of spruce cpDNA continued. and mapping of the chloroplast genome was initiated. Studies using RFLP analysis to determine the inheritance pattern of mtDNA in the spruces were also initiated.

Publications: 88/01 to 88/12

- DAVIS, J.M. and KEATHLEY, D.E. 1987. In vitro propagation of mature black locust (Robinia pseudoacacia L.). Plant Cell Reports 6:431-434.
- DAVIS, J.M. and KEATHLEY, D.E. 1988. In vitro propagation of mature Robinia pseudoacacia L. using bud explants obtained during winter domarncy. In: The Genetic Manipulation of Woody plants, J.W. Hanover and D.E. Keathley Eds.
- STINE, M and KEATHLEY, D.E. 1988. Evidence for paternal inheritance of plastids in interspecific hybrids of Picea. In:The Genetic Manipulation of Woody Plants, J.W. Hanover and D.E. Keathley Eds. Plenum Press, New York. p 486. (Abstract).

 DAVIS, J.M. and KEATHLEY, D.E. 1988. In vitro
- DAVIS, J.M. and KEATHLEY, D.E. 1988. In vitro propagation of a black locust tree with an unusual phenotype. Nitrogen Fixing Tree Research Reports 6:65-67.
- HAN, K.H. and KEATHLEY, D.E. 1988. Isolation and culture of protoplasts from callus tissue of black locust (Robinia pseudoacacia L.). Nitrogen Fixing Tree Research Reports 6:68-70.
- ERNST, S., HANOVER, J.W. and KEATHLEY, D.E. 1988. Genetic variation and control of intraspecific crossability in blue and Engelman spruce. Silvae Genetica 37:112-117.

O6.009 CRISO010920 GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES

ECKERT R T; Forest Resources; University of New Hampshire, Ourham, **NEW HAMPSHIRE** 03824. Proj. No.: NH00179 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 91

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Objectives: Develop strategies for genetic selection with particular emphasis on juvenile growth and resistance to disease, cold and drought. Develop and implement strategies for the effective propagation and deployment of genetically selected timber, urban and Christmas trees.

Approach: Isozyme methodology will be developed for resolving genic-level variation in trees. Emphasis will be placed on members of the Fagaceae and Pinaceae due to their economic importance, but members of other families may be included. Analyses of growth will follow a molecular approach. Relationships between extracted chloroplast performance, under specific experimental conditions, and chlorophyll DNA variation will be explored. The potential for selecting high performance families based on restriction fragment length polymorphisms will be explored. Potential breeding zones for eastern white pine will be identified based on multilocus allozyme variation. Potential breeding zones based on performance of progeny tests will be compared with the zones suggested by the multilocus information. Recommendations will be made for breeding zones based on the outcome of this process.

Progress: 88/01 to 88/12. Eight stands of Atlantic white-cedar Chamaecyparis thyoides (L.B.S.P.) at the northern margin of the species range were sampled in New Hampshire and Maine for studies of genetic variability based on isozyme analysis of foliage. Thirty-four alleles at 16 loci were assayed from a total of 160 individual trees. Chi-square analysis and F(st) estimates support an interpretation that substantial genetic variation exists among stands. Heterozygosity varied substantially among stands, averaging 12%. Patterns of genetic variation displayed along canonical axes illustrate that populations are truly different from one another and that at least one stand is unique, in that it contains alleles not found in the other stands. The distribution of genetic variation is independent of geographic location at the scale of this study. Adjacent stands may be substantially different in genetic composition. This type of research has significance in the study of potential effects on forest tree species due to global climate change. This approach plays a role in identifying populations which contain high levels of genetic variation, which could become important centers for dispersion during climate change. Marginal populations have special significance for adaptation to climate change. Protection plans may need to be developed for high value populations.

Publications: 88/01 to 88/12

STEINER, K.C., WILLIAMS, M.W., OEHAYES,D.H., HALL, R.B., ECKERT, R.T., BAGLEY, W.T., LEMMIEN, W.A., KARNOSKY, O.F., CARTER, K.K. and CECH, F.C. 1988. Juvenile performance in a range-wide provenance test of Fraxinus pennsylvanica Marsh.

O6.010 CRISO137977 ISOLATION OF A LIGNIN-BIOSYNTHESIS GENE FROM LOBLOLLY PINE

SEOEROFF R R; CHANG H; STOMP A; College of Forest Resources; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO9405 Project Type: CRGO Agency ID: CRGO Period: 15 SEP 88 to 30 SEP 90

Objectives: PROJ. 8800494. We have begun a long-term program on genetic modification of lignin biosynthesis in loblolly pine that rests on a multi-disciplinary approach, including enzyme biochemistry, recombinant ONA techniques, ONA transfer methodology, lignin chemistry, and testing of modified plants under field conditions. To begin this work, we have developed methods for the isolation transfer and expression of specific genes in pines (Sederoff et. al. 1986; Stomp et. al. 1987; Kinlaw et. al. 1987; Harry et. al. 1987). We now have the basic technology to approach the modification of lignin biosynthesis.

Approach: Our approach is to focus on two objectives: 1) isolate genes that could be used to modify the biosynthesis of lignin, and 2) isolate genetic regulatory elements that are responsible for the expression of specific genes during lignin biosynthesis. Using these genes and the proper regulatory elements, we could begin to construct genes and modify enzyme coding sequences to affect the rate, quantity or kind of lignin that is produced in loblolly pine.

O6.011 CRISO130310 CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA DNA

CULLIS C A: Biology; Western Reserve University, Cleveland, **OHIO** 44106.
Proj. No.: OHOR-8602457 Project Type: CRGO Agency IO: CRGO Period: 15 SEP 86 to 30 SEP 88

Objectives: PROJECT 8602457. To clone and characterize the 25S, 18S and 5S ribosomal RNA genes from Pinus radiata. To use these and other cloned probes to evaluate quantitative and qualitative polymorphisms in natural populations.

Approach: The rONAs will be cloned from gradient enriched fractions. Other repeated DNA sequences will be selected from a pine genomic library in the phage vector EMBL4. The probes will be used on ONAs from individual trees from a range of provenances. The quantitative and qualitative variation will be assessed using slot blotting and Southern hybridizations.

Chromosomal localization will be attempted using in situ hybridization.

Progress: 86/09 to 88/09. A characterization of the variability of the Pinus radiata nuclear DNA has been started using the repetitive sequence fraction. The rDNA (that coding for the 25S and 18S ribosomal RNAs) and the 5S DNA have been characterized, as well as a number of other highly repetitive sequences in the P. radiata genome. The rDNA was localized using in situ hybridization, and shown to be present on a number of chromosomes, at both major and minor sites. Comparisons between individuals demonstrated quantitative and qualitative heterogeneity for this class of genes. This family of genes should be sufficient to unambiguously identify six of the linkage groups. Isolation of clones containing rDNA from a library has demonstrated this heterogeniety also exists at the sequence level, making the pine rDNA somewhat different from the previously described angiosperm rDNAs. The 5S DNA is dispersed, but more homogeneous than the rDNA. Nineteen other repetitive sequence families have been isolated and these appear more conserved between individuals than the rDNA. However, sufficient polymorphisms exist in some of these families to allow their use as RFLP markers. Two heterologous probes which have potential for use as RFLP markers are the M13 hypervariable sequence and the human 33.6 probe. Both of these hybridize to P. radiata DNA with a discernible banding pattern. The set of probes already characterized may be sufficient to deliniate the twelve linkage groups in this species.

Publications: 86/09 to 88/09

CULLIS, C.A., CREISSEN, G.P., GORMAN, S.W. and TEASDALE, R.D. 1988. The 25S, 18S, and 5S ribosomal RNA genes from Pinus radiat D. Don Molecular Genetics of Forest Trees Petawawa National Forestry Institute Information Report.

06.012 CRISO130207 CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM

PRIBNOW D G; GOLD M H; Oregon Graduate Center, Beaverton, $\mathbf{OREGON} = 97006$.

Proj. No.: ORER-8602787 Project Type: CRGO Agency ID: CRGO Period: 30 SEP 86 to 30 NOV 89

Objectives: PROJ 8602787. In order to enhance the potential of Phanerochaete chrysosporium for lignin biodegradation, we need to understand the molecular structure and mechanism of expression of the genes coding for the enzymic components of the degradative system. It is now clear that two P. chrysosporium enzymes, lignin peroxidase (LiP) and manganese peroxidase (MnP), constitute important components of the degradation system. Hence, initially we intend to study the molecular structure and regulation of the genes encoding these enzymes.

Approach: The following experimental approach will be taken. First, using synthetic oligonucleotide probes, assays based on enzyme

function, or specific antibody probes we will isolate LiP and MnP genes from genomic and cDNA libraries prepared from P. chrysosporium. Then we will determine the DNA sequences of the structural genes and their flanking regions for both enzymes in order to predict amino acid sequences for the two enzymes and also in order to ascertain important structural features of the genes themselves, including regulatory signals and intron-exon boundaries.

Progress: 87/01 to 87/12. Purified lignin peroxidase (LiP) and manganese peroxidase (MnP) were used to raise polyclonal antibodies in rabbits. These antibodies were then used to screen a bacteriophage lambda gt11 library for recombinant phages carrying fungal cDNA's for these enzymes. A second anti-rabbit-antibody complexed to alkaline phosphatase was used to detect positive clones. More than twenty independent clones were isolated for each of the peroxidases. Lambda DNA was isolated from each clone and the size of each insert was determined to be between 0.6-1.3 Kb. Concurrently, the N-terminal amino acid sequences were determined for purified LiP and MnP proteins. Oligonucleotide probes based on the N-terminal sequences were prepared and used to confirm the identities of clones selected by antibody screening. Several of the large lambda gt11 inserts which hybridize to the oligonucleotide probes will be ligated into a suitable sequencing vector such as M13mp18 or pIBI30 in preparation for sequencing the entire coding regions of the LiP and MnP genes. These cDNA clones will also be used to probe a genomic DNA library in order to investigate the extent and size of the postulated gene families coding for LiP and MnP isozymes.

Publications: 87/01 to 87/12
 NO PUBLICATIONS REPORTED THIS PERIOD.

O6.013 CRISO081168 PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF DOUGLAS-FIR IN SOUTHERN OREGON

ADAMS W T; Forest Science; Oregon State
University, Corvallis, **OREGON** 97331.

Proj. No.: ORE-FS-115-R Project Type: STATE
Agency ID: OCI Period: O1 JAN 80 to 30 JUN 86

Objectives: Determine the relative influence of the various evolutionary forces on patterns of genetic variation of Douglas-fir in southern Oregon and assess the usefulness of allozymes for certifying seedlots.

Approach: The allozyme genotypes of 50 or more parent trees in each of 22 breeding zones will be determined by electrophoretic analysis of their seeds. Comparison of allozyme variation patterns with expectations based on population genetic models will allow assessment of the relative influence of different evolutionary mechanisms. The allozyme composition of seedlots from several seed zones will also be determined. The value of allozymes for seed certification will depend on the ability to distinguish seedlots based on their allozyme compositions.

Progress: 80/01 to 86/06. Population genetic structure of Douglas-fir and Jeffrey pine in southwest Oregon were investigated using quantitative traits evaluated in seedling common garden studies and simply inherited genetic markers (allozymes). It was hoped that allozymes would provide a time-saving alternative to longer-term seedling and field studies as a means to map adaptive variation and provide information for designing provisional seed transfer rules and breeding zones. Although allozymes proved useful for describing patterns of genetic variation on large geographical scale, little useful allozyme variation was detectable within the restricted geographical region of southwest Oregon. Allozymes did prove valuable for studies on mating systems and gene flow in natural populations; and thus, can be an important tool for the forest geneticist. Almost all seedling traits investigated showed a great deal of genetic variation, both within and among populations in the region. Growth rhythm traits are of particular interest because a proper fit to growing season is essential for the avoidance of damage from frost or drought. Shoot growth rhythm in Douglas-fir was found to be finely tuned to local climate and needs to be carefully considered in tree breeding programs so as to maximize both adaptation and growth. One manuscript has been submitted for publication and three will be written and submitted for publication. Six Graduate Students Associated.

Publications: 80/01 to 86/06

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FURNIER, G.R. and ADAMS, W.T. 1986. Mating system in natural populations of Jeffrey Pine. Amer. J. Bot. 73(7):1002-1008.

FURNIER, G.R. and ADAMS, W.T. 1986. Geographic patterns of allozyme variation in Jeffrey pine. Amer. J. Bot. 73(7):1009-1015.

LI, P. 1986. Rangewide patterns of allozyme variation in Douglas-fir (Pseudotsuga Menziesii (Mirb.) Franco). M.S. thesis, Oregon State University, Corvallis.

NEALE, D.B. 1985. Genetic implications of shelterwood regeneration of Douglas-fir in southwest Oregon. Forest Sci. 31:995-105.

NEALE, D.B. and ADAMS, W.T. 1985. The mating system in natural and shelterwood stands of Douglas-fir. Theor. Appl. Genet. 71:201-207.

NEALE, D.B. and ADAMS, W.T. 1985. Allozyme and mating-system variation in balsam fir (Abies balsamea) across a continuous elevational transect. Can. J. Bot. 63:2448-2453.

O6.014 CRISOOO0288 GENETIC IMPROVEMENT OF NORTHWEST TREES

SILEN R R; Forestry Sciences Laboratory; Pacific NW Forest & Rge Exp Sta, Corvallis, OREGON 97331.

Proj. No.: PNW-1401 Project Type: INHOUSE Agency ID: FS Period: 19 AUG 85 to 19 AUG 90

Objectives: To safely yet substantially improve growth, form, and resistances of Northwest trees through genetic research.

Approach: Some techniques are still inadequate to overcome technical problems of tree breeding programs. Adequate advanced-generation breeding information is lacking for Northwest species. Criteria for delimiting safe seed movement is inadequate.

Progress: 85/01 to 85/12. High density tree sampling has permitted generation of high resolution genetic maps of local height variation. Genetic maps enable geneticists to maximize genetic gain and minimize loss due to inappropriate seed transfers. Regionwide estimates of genetic parameters are now provided for 17 breeding zones. Height gains average about 5 percent. Low individual tree and high family heritability indicate new parent tree tests must be made of each breeding generation. Results from 15-year-old progeny tests indicate 1:3 relationship between height and volume superiority. The importance of this relationship is that while most early computations of gain are made from height data, forestry managers prefer volume information. A multivariate analysis procedure utilizing geographic and climatic variables in a model which predicts fitness of a population when used at a different planting site. Use of the model will reduce improper seed movement. In western hemlock, 8- to 10-percent gains in first-year height and 20- to 30-percent gains for volume are possible when rooted cuttings are used rather than seedlings.

Publications: 85/01 to 85/12

- SILEN, R.R. 1984. High resolution mapping of inherent growth variation in coastal Douglas-fir. In: progeny testing: Proceedings of Servicewide Genetic Workshop; 1983 December 5-9, Charleston, SC, Washington, D.C. USDA, For. Serv., 581-597
- SILEN, R.R. 1985. Selection differentials, heritabilities, and gains from Cooperative progeny test data. Indust. For. Assoc., Tree Improvement Newsletter No. 47. 8-9.
- SILEN, R.R. 1985. Volume superiority and gain estimates from progeny test data. Indust. For. Assoc., Tree Improvement Newsletter No. 48; 4-6.
- CAMPBELL, R.K. 1984. Procedures for determining the biological limits of breeding zones in the Pacific Northwest. In: Progeny testing: Proceedings of Servicewide Genetic Workshop; 1983 December 5-9, Charleston, SC., Washington, D.C.
- FOSTER, G.S., CAMPBELL, R.K. and ADAMS, W.T. 1984. Heritability, gain and C effects in rooting of western hemlock cuttings. Can. J. of For. Res.

14(5):628-638, p. 488-493.

FOSTER, G.S., CAMPBELL, R.K. and ADAMS, W.T. 1984. Clonal selection prospects in western hemlock combining rooting traits with juvenile height growth. Can. J. of For. Res. 15:488-493.

O6.015 CRISO094043
ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT
SYSTEMS

YENDOL W G; MCCARTHY W J; Entomology; Pennsylvania State University, University Park, PENNSYLVANIA 16802.

Proj. No.: PENO2757 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 89

Objectives: To determine and analyze the physical and biotic factors that regulate epizootics of entomopathogens.

Approach: Gypsy moth larvae will be subjected to biotic and abiotic stressors. Bioassays will be used to determine changes in susceptibility of stressed larvae to NPV or Bt. Abiotic factors and biotic factors will be studied. Physiological, biochemical and immunological characteristics of the larvae will be examined by measuring changes in gut pH, total hemolyte count, normal bacterial flora and other parameters and also possible changes in microorganism. Various strategies will be employed to construct a physical map of a cloned gypsy moth virus genome. An improved cell culture line (the Ld652Y line from Beltsville) will be used. Gypsy moth virus isolates, differing in biological activity, will be analyzed to identify regions of genome differences and then mapped. As genes are identified (i.e., polyhedrin gene, etc.) the position on the map will be determined.

Progress: 88/01 to 88/12. Three distinct clonal variants of the gypsy moth nuclearpolyhedrosis virus (LdMNPV) have been evaluated for their potential as biological control agents. The wild type Hamden population (LDP-67) was subjected to double plaque purification in IPLB-LD-652Y cells, resulting in three distinguishable classes of variants, based on the number of PIBs produced per cell 'in vitro'. Representatives were chosen from each variant class and redesignated accordingly; Ld-S (several PIBs/cell), Ld-F (few PIBs/cell), Ld-V (variable numbers of PIB/s cell). These representative variants were compared to the wild type by restriction enzyme analysis and by bioassay in 'L. dispar' larvae. With all enzymes tested the variants demonstrated similar but different restriction patterns. The molecular weights of the three variants were estimated at 159-163 Kb. In bioassay trials two of the variants, Ld-V and Ld-S, exhibited LD(50) values approximately 3.9 times less than the LD(50) from the wild type population. The Ld-F variant was not infective in feeding trials. The stability of one variant, Ld-S, was tested by high multiplicity passage (HMP) in IPLB-LD-652Y cells. By the 20th undiluted passage, the 'in vitro' CPE of this variant was significantly altered. DNA isolated from the P-20 stock exhibited several differences in the restriction endonuclease (REN) profile relative to the early passage virus. Genomic alterations were more clearly visualized after plaquing.

Publications: 88/01 to 88/12
KEATING, S. T., MCCARTHY, W. J., YENDOL, W.
G. Gypsy moth ('Lymantria dispar') larval
susceptibility to a baculovirus affected by

selected nutrients, hydrogen ions (pH) and plant allelochemicals in artificial diets. J. Invert. Pathol. (in press).

O6.016
CYTOGENETIC STUDIES OF HARDWOOD AND CONIFEROUS
FOREST TREES

SCHLARBAUM S E; Forestry Fisheries & Wildlife; University of Tennessee, Knoxville, **TENNESSEE** 37996.

Proj. No.: TENOOO44-MS

Project Type: MCINTIRE-STENNIS Agency ID: CSRS Period: O1 DEC 84 to 30 SEP 89

Objectives: Evaluate different procedures for chromosome studies of forest trees using uniform-staining reagents. Evaluate and refine chromosome banding procedures for coniferous and hardwood trees. Investigate methodology for somatic nucleolar staining and relate to ploidy level in tree species. Conduct karyological analyses of tree species for use in chromosome mediated gene transfer experiments, gene mapping, and determination of cytotaxonomic and phylogenetic relationships.

Approach: Different cytological procedures will be used for mitotic and meiotic chromosomes to determine optimum: material collection time, pretreatment reagent and length, and fixative. Allocycly and G-, N-, and Q-banding procedures will be contrasted to identify the best procedure for specific chromosome identification. Different nucleoli staining procedures will be evaluated and reliability of nucleoli number to ploidy level will be determined. Karyotype analyses will beused to identify chromosome additions in recipient cell calli. Karyotypes will be synthesized with other taxonomic information for determination of cytotaxonomic and phylogenetic relationships.

Progress: 88/01 to 88/12. Research on banding coniferous tree chromosomes was conducted. Studies on inducing G-bands in conifer chromosomes using a trypsin digest followed by Giemsa staining were not successful as in previous studies. Banding of chromosomes using mammalian protocols involving restriction endonuclease enzymes followed by Giemsa staining were not successful. Results from published molecular research indicate that the highly repeated DNA sequences in conifers are distributed throughout the genome rather than concentrated in various locations. This research explains the negative results of the protocols involving restriction endonucleases in which extensive cleavage of concentrated highly repeated DNA sequences is a requirement for banding. Banding of chromosomes was observed in control slides (no special treatments) of both of the above banding protocols. The single published report of G-banding shows only several banded chromosomes which may or may not have banded due to the trypsin digest. No further attempts will be made to G-band chromosomes using trypsin digests. Future attempts will involve fluorescence and in situ hybridization approaches. Graduate students - 0.

Publications: 88/01 to 88/12

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SCHLARBAUM, S.E. (1988). Somatic cell genetics in forestry: intergration of cytogenetics, tissue culture and molecular genetics. In Somatic Cell Genetics in Forestry. M. R. Ahuja, ed. Martinus Nijhoff Pub., Netherlands. p.103-118.

06.017 CRISO137239 MOLECULAR BIOLOGY OF CELLULOSE AND LIGNIN DEGRADATION BY PHANEROCHAETE CHRYSOSPORIUM

CULLEN D; KERSTEN P J; USDA Forest Service; One Gifford PinchOt Drive, Madison, **WISCONSIN** 53705.

Proj. No.: WISR-8802581 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 88 to 30 SEP 91

Objectives: Proj. 8802581. The overall objectives of this proposal are: A) to elucidate the role of specific enzymes in wood decay, and B) to improve the biopulping performance of Phanerochaete chrysosporium. Our specific objectives are the following: 1) determine number, structure, and organization of the genes encoding cellulases and glyoxal oxidase; 2) establish the role of individual cellulase. ligninase, and glyoxal oxidase genes in delignification; and 3) assess the biopulping performance of transformants with inactivated and/or altered gene expression.

Approach: Using standard recombinant DNA techniques, cellulases and glyoxal oxidase genes will be cloned and sequenced. Appropriate vectors will be constructed and cellulase, ligninase, and glyoxal oxidase gene(s) will be selectively disrupted. The enzyme profiles and delignification abilities of these recombinant P. chrysosporium strains will then be assessed. Promising transformants will be evaluated in large scale biomechanical pulping trials.

06.018 CRISO097400 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY

JOHNSON J; ROTH D; BULLA L; Molecular Biology; University of Wyoming, Laramie, WYOMING 82070. Proj. No.: WYO-224-86 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To isolate and to characterize genes in beneficial and pathogenic microbes controlling nitrogen fixation, biological control of pathogens and weeds, host-parasite interactions, host specificity and hypovirulence.

Approach: Although research groups participating in this project are working on different problems and with different organisms, they are all using the same tools of modern molecular genetics and recombinant DNA technology. Before discussing specific procedures which will be used to clone and to characterize genes in plants and associated microbes, it would be useful to briefly discuss

the potential and limitations of some of these procedures and techniques.

Progress: 88/01 to 88/12. All projects being carried out have shown substantial progress. Johnson: Frankia N(subscript 2) Fixation-Fragments of Frankia DNA which activate transcription of a LUX cassete have been identified and DNA sequence analyses being done to identify promoter elements. RothJohnson; antisense RNA inhibition of plant virus. Sequences complementary to the 5' region of TMV have been tested in vitro and in vivo for the ability to interfere with TMV gene expression. Roth; TMV replicase activity/protein kinase activities. A unique protein kinase induced by plant viroids has been identified. Purification and characterization of TMV induced replicase is in progress. Bulla-characterization of B. thuringiensis insecticidal proteins. The genes encoding the insecticidal activity are being cloned into the blue-green algae Anacystis nidulans to develop a self-perpetuating biological insecticide effective against insect vectors of disease.

Publications: 88/01 to 88/12
 CRUM, C.J., JOHNSON, J., NELSON, A. and ROTH,
 D. (1988). Nucl. Acids Res. 16, 4569-4581.
 HIDDINGA, H.J., CRUM, C.J., HU, J., and ROTH,
 D. (1988). Science 241, 451-453.

CM 07 RANGE

O7.001 CRISO097028
GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY

SANDS D C; BLAKE T K; Plant Pathology; Montana State University, Bozeman, MONTANA 59717.

Proj. No.: MONBOO231 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 90

Objectives: To isolate and characterize genes in plants controlling development, metabolism and resistance to pathogens. To isolate and characterize genes in beneficial and pathogenic microbes controlling biological control of weeds.

Approach: Using wheat, barley and Sclerotinia-genetic systems will be studied using cell culture, mutagenesis, and DNA methods including recombinant libraries of DNA and their analysis with radioactive probes of CDNA. Restriction mapping will also be used in restriction fragment length polymorphism studies of barley. The barley - Xanthomonas host parasite system will be investigated with these methods. Host range reduction of Sclerotinia will be attempted, for applications in biocontrol of weeds.

Progress: 88/01 to 88/12. Several mutants of Sclerotinia sclerotiorum have been obtained by ultraviolet light-8-methoxy-psoralin treatment. These mutants are restricted in host range, or are auxotrophic, or are sclerotialess. In each case they may be of value as weed pathogens because of their limitation in terms of spread beyond the target host. In the past year we have found that protoplasts can be made, and regenerated from this fungus, and that their nuclear number ranges from one to more than ten. Uninucleate protoplasts would be useful for mutation experiments where auxotrophs and other recessive characteristics are desired.

Publications: 88/01 to 88/12
ZIDACK, N. K., FORD, E., HENSON, J. and
 SANDS, D. C. 1988. Uninucleate protoplasts
 of Sclerotinia sclerotiorum for genetic
 manipulation. APS Abstracts 297.

CM 09 CITRUS AND SUBTROPICAL FRUIT

09.001 CRISO142123
MOLECULAR BIOLOGY OF PLANT SENESCENCE

THEOLOGIS A; TBD; TBD; Agricultural Research Service, Albany, **CALIFORNIA** 94710.

Proj. No.: 5335-23410-002-00D

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Project Type: INHOUSE Agency ID: ARS Period: 15 JUL 87 to 31 JAN 88

Objectives: Isolate & structurally characterize genomic sequences to early auxin- inducible mRNAs from pea tissue. Regulate expression of the hormonally regulated genes in vivo & in isolated pea nuclei. Purify & localize at the subcellular level the proteins coded by the auxin-regulated genes.

Approach: Genomic libraries will be constructed into the cloning vector EMBL 3 using DNA from etiolated pea seedlings. The genomic sequences of the early auxin regulated mRNAs will be isolated by plaque filter hybridization using the already isolated cDNA clones pIAA4/5 & pIAA6 as probes. The organization & structural analysis of the genes will be investigated in detail. To deter- mine whether auxin acts at the transcriptional or post-transcriptional level, the stability of the inducible mRNAs will be examined in vivo. Theseexperiments will be supplemented with in vitro transcription in isolated nuclei. Finally, the auxin cDNA clones will be introduced into gamma expression vectors to produce fused proteins with beta-galactosidase. Anti-bodies directed toward the hybrid proteins will then be used to purify the proteins, localize them at the subcellular level & determine the kinetics of their accumulation during cell growth.

Progress: 88/01 to 88/12. During the last year we were able to isolate a DNA sequence complementary to the mRNA of Acc Synthase in Cucurbita Fruit. The experimental approach used was unique and allowed us to clone the Acc synthase rapidly. The experimental evidence indicates that the Acc Synthase gene is transcriptionally regulated by auxin and Li ions. More recently we were able to isolate genomic sequences to Cucurbita as well as to tomato Acc synthase. Structural characaterization of the genes is in progress.

Publications: 88/01 to 88/12

THEOLOGIS, A. 1988. Regulation of Gene Expression by Idoleacetic Acid in Pea Epicotyl Tissue. In the Biomech. Reg. Growth & Dev. Keys to Prog. Beltsville Sym XII. Editors, Steffens, GL and Rumsey TS. Kluwer Academic Publisher.

THEOLOGIS, A. 1988. Auxin Reg. Gene
Expression in Pea. In "Plant Biotechnology"
Editors, Kung, SD and Arntzen, CJ.
Publishers: Butterworths. To appear in the
Buttersworths Biotech. Series. J.Davies,
Biogen Series Editor. In Press.

THEOLOGIS, A. 1988. Molecular Cloning of Early IAA Reg. mRNA in Pea. Proceedings of the Intnl. Symp. on "The Physiology and Bioch. of Auxins in Plants", Liblice, Czechoslovakia, 09/28-10/2/87. Editors: Kutacek, M., Bandurski, R. and Kerkula REDDY, S., KOSHIBA, T., THEOLOGIS, A. and POOVIAH, B.W. 1988. The Effect of Calcium Antagonists on Auxin-Induced Elongation and

on the Expression of Two Auxin-Regulated Genes in Pea Epicotyls. Plant Cell Physiology 29: 1165-1170.

SATO, T. and THEOLOGIS, A. 1988. Cloning the mRNA of ACC Synthase: The key regulatory enzyme in the Ethylene Biosynthetic Pathaway in Plants: Scienc.

SATO, T. and THEOLOGIS, A. 1988. ACC Synthase from Cucurbita: Purification Properties and Antibody Production. Submitted to J.B.C.

09.002 CRISO088642 CELLULAR GENETICS OF CITRUS SPECIES

MOORE G A; Fruit Crops; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-FRC-02091 Project Type: HATCH Agency ID: CSRS Period: 12 OCT 82 to 30 SEP 86

Objectives: Develop cellular genetic techniques to be used in conjunction with conventional genetic, cytogenetic, and plant breeding approaches to Citrus improvement.

Approach: Regenerate plants from embryogenic or organogenic tissue cultures of citrus genotypes, characterize the chromosome numbers and morphologies of the plants using standard cytogenetic techniques, determine the isozyme banding patterns of the plants, use these techniques along with plant morphologies to determine the inherent variability in citrus species in vivo and in vitro.

Progress: 85/10 to 86/10. Research on Citrus and Prunus isozyme systems is continuing, and studies are also underway to identify and characterize isozyme loci in Ananas and Vitis. When isozymes were used to characterize open-pollinated Citrus rootstock populations, some populations were found to consist solely of nucellar seedlings, while a few populations contained relatively high (approximately 20%) numbers of zygotic seedlings. Field studies are planned to verify and expand on these observations. Initial steps in developing a system for the examination of restriction fragment length polymorphisms in Citrus are underway. Tissue culture studies are also being performed. Factors that may increase morphogenesis in 'Hamlin' orange, 'Key' lime, and 'Carrizo' citrange cultures are being evaluated. A method for rapid in vitro propagation of Ananas is being developed, and the genetic stability of regenerated pineapple plants is being studied.

Publications: 85/10 to 86/10

MOORE, G.A. 1986. In vitro production of Citrus rootstocks. HortScience 21:300-301.

DEWALD, S.G., and MOORE, G.A. Somaclonal variation as a tool for the improvement of perennial fruit crops. Accepted for pub., Fruit Varieties J.

DURHAM, R.E. 1986. Isozyme banding patterns and inheritance studies in peach (Prunus persica (L.) Batsch). M.S. Thesis. University of Florida, 63 pages.

09.003 CRISO142536 CHARACTERIZATION OF MUSA CLONES AND SPECIES USING ISOZYMES AND ORGANELLE DNA MARKERS

JARRET R L; JOHNSON C; Horticulture; University of Georgia, Athens, **GEORGIA** 30602. Proj. No.: 6607-21000-001-055

Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: O1 FEB 88 to 31 JAN 90

Objectives: To clarify the taxonomic relationship of Musa species, to examine their evolutionary relationship with cultivated bananas and plaintains and to utilize genetic/biochemical markers for clonal identification. The marker systems to be employed will include a range of isozyme markers in conjunction with organelle RFLPs. The data will be compared with that collected via standard morphological analysis.

Approach: Plant material (leaf) from Musa species, now in germplasm collections around the world, will be collected and frozen (-135 degree). Samples will be analyzed for enzyme polymorphisms and organelle DNA RFLPs using techniques developed by R. Jarret. Data collected will be numerically analyzed and compared with recent morphological data on same plant material. Data will be used to define systematic relationships and for clonal identification.

Progress: 88/02 to 88/12. A post-doctoral fellow was hired in December 1988 to initiate work on this project. To date, a cDNA library has been constructed for use with this genus using a malaccensis subspecies. DNA sequences have been isolated and cloned in E. coli. Present activities are focused on the use of biotin-labelled probes for the detection of single and low-copy number sequences in total DNA extracts from various clones.

Publications: 88/02 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

O9.004 CRISO009070
PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF
TROPICAL FRUIT AND THEIR CONTROL

PATIL S S; Plant Pathology; University of Hawaii, Honolulu, **HAWAII** 96822.

Proj. No.: HAWO0717 Project Type: HATCH Agency ID: CSRS Period: O1 FEB 83 to 30 SEP 86

Objectives: To find an "antipenetrant" which effectively prevents C. gloeosporioides infection of papayas in the field. To construct, using recombinant DNA technology, an epiphytic bacterium which produces anticutinase. To determine the role of cutinases in the field infection of papayas by other papaya post-harvest pathogens.

Approach: Field sprays involving the currently registered commercially available organophosphorus pesticide will be conducted to determine the most efficacious compound that will prevent papaya infection by C. gloeosporioides. Monoclonal antibodies to C. gloeosporiodes cutinase will be developed and

mRNA which encodes such antibodies will be isolated, C DNA will be made from the mRNA, and it will be introduced into an epiphytic bacterium. The bacterium should be capable of producing anticutinase in the field to stop C. gloeosporioides infection of papayas. Cutinases of other post-harvest fungal pathogens of papayas will be studied.

Progress: 83/10 to 86/09. physiological, biochemical and mutational studies have shown that cutinase secreted by the fungus Colletotrichum gloeosporioides Penz., the casual agent of papaya anthracnose. is required for pathogen ingress in unwounded papaya tissues. Using the techniques of recombinant DNA, we have isolated several clones from the library made from the genomic DNA of the pathogen which are induced by cutin. Nine of these clones showed hybridization to specific regions in the cutin-growth mRNA, but no hybridization to the glucose-grown mRNA. The pattern of bands indicated that several different transcripts are present in cutin-grown cells that are absent in glucose-grown cells. Thus, these clones may contain sequences encoding cutinase and/or other related transcripts induced by cutin. DNA from the nine clones was digested with HindIII. Results indicated that transcripts arise from at least three different regions of the C. gloeosporioides genome. To isolate the cutinase encoding sequence, the three clones were screened by hybrid selection of cutin and glucose grown mRNA, in vitro translation of the message and immunoprecipitation of the product. Results showed that two clones have sequences which encode for products precipitable by rabbit anticutinase.

Publications: 83/10 to 86/09

DICKMAN, M.B. and PATIL, S.S. 1986. A rapid and sensitive plate assay for the detection of cutiase produced by plant pathogenic fungi. Phytopathology. 76:475-476.

DICKMAN, M.B. and PATIL, S.S. 1986. Cutinase

DICKMAN, M.B. and PATIL, S.S. 1986. Cutinase deficient mutants of Colletotrichum gloeosporioides are nonpathogenic to papaya fruit. Physiological Plant Pathology. 28:235-242.

DICKMAN, M.B. and PATIL, S.S. 1986. Isolation of DNA sequences from Colletotrichum gloeosporioides preferentially expressed when induced by cutin.

Journal of Cellular Biochemistry. Supplement 10c, Alan R. Liss, Inc., New York. p. 151.

O9.005 CRISO001941
GENETIC MANIPULATIONS AND GENE EXPRESSION IN
WOODY PERENNIAL PLANTS.

KORBAN S S; Horticulture; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.

Proj. No.: ILLU-65-0325 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 91

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Objectives: Combine genetic immunity to apple scab, powdery mildew, cedar-apple rust and fireblight into enhanced germplasm. Study the genetic dwarf character in Malus and incorporate it into enhanced germplasm. Investigate gene regulation and expression at

the molecular level in plants and determine their behavior during plant development. Manipulate the plant genome through transformation and establish novel methods for identifying transformants. Establish cell/tissue culture protocols to induce and screen stable genetic variants.

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Approach: Controlled crosses will be made among advanced apple germplasm lines to combine genes for disease resistance. Sexual hybridization will be used to study the genetic dwarf character; electrophoretic and spectrophotometric techniques will be used to characterize the gene product(s) for this trait. Techniques will be established for in vitro screening for various diseases, dwarfism, and cold hardiness using callus and cell cultures of apple, pear, peach and other woody plants. Regulatory sequences of genes encoding for LHCP II gene will be characterized and sequenced; transcription initiation site will be investigated, as well as the presence of enhancer-like elements. Chimeric genes will be constructed and used to establish transgenic plants.

Progress: 86/10 to 87/09. To identify and isolate light-harvesting chlorophyll a/b binding polypeptide (LHCP II) genes from the apple genomic library, a pea LHCP-cDNA was labeled by nick-translation and used to probe the library that was constructed earlier in our laboratory. Fifteen positive clones containing apple LHCP genes were recovered. On the basis of the restriction pattern and southern blots, an apple LHCP II gene was localized within a 14Kb insert of recombinant Charon 35 DNA. This LHCP gene has been subcloned into an M13 mp18 RF DNA vector. Clones harboring recombinant M13 DNA containing the LHCP gene were identified by color development on indicator plates and by colony hybridization. The structure of this gene is being analyzed using Sanger's method. Screening shoot tip cultures of five apple genotypes using a hypodermic needle inoculation for fire blight infection resulted in the development of shoot necrosis in most genotypes; however, no distinction in the susceptibility of the genotypes for fire blight infection was observed. Therefore, new fire blight inoculation techniques are presently under investigation. Apple callus cultures of stem sections derived from in vitro-grown shoots were successfully established and will be induced to regenerate shoots. Seven scab resistant apple seedlings have been selected in the field based on their high fruit quality and outstanding horticultural characteristics. Two new scab resistant apple cultivars have been named and released.

Publications: 86/10 to 87/09

CHEN, H. and KORBAN, S.S. 1987. Genetic variability, and the inheritance of resistance to cedar-apple rust in apple. Plant Pathology 36:168-174.

KORBAN, S.S., CHEN, H. and RIES, S.M. 1987. Interaction of apple cultivars with populations of cedar-apple rust. Jour. of Phytopath. 119:272-278.

BOURNIVAL, B.L. and KORBAN, S.S. 1987. Electrophoretic analysis of genetic variability in the apple. Scientia Hortic. 31:233-243.

KORBAN, S.S. and BOURNIVAL, B.L. 1987. Catalase, esterase, and peroxidase enzymes in seeds and leaves of Malus Xdomestica Borkh. Scientia Hortic. 32:213-219.

JOUNG, H., KORBAN, S.S. and SKIRVIN, R.M. 1987. Screening Malus shoot cultures for cedar-apple rust infection using in vitro inoculation. Plant Disease 71:(IN PRESS).

CHEN, H., ERDOS, G., KORBAN, S.S. and BEUTOW, D.E. 1987. Isolation of LHCP (II) genes from an apple genomic library. Plant Physiol. 83(4):18.

CHEN, H., KORBIN, S.S., BUETOW, D.E. and ERDOS, G. 1987. Characterization of apple LHCP genes. HortScience 22:1070.

O9.006 CRISO141958 PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAD DISEASES OF FRUIT TREES

CIVEROLO E L; Beltsville Agr Res Center, Beltsville, MARYLAND 20705. Proj. No.: 1275-24000-052-00D

Project Type: INHOUSE Agency ID: ARS Period: 22 AUG 86 to 21 AUG 91

Objectives: (1) Study pathogenecity and develop improved disease diagnostic and identification techniques for Xanthomonads; (2) identify genes affecting virulence and host recognition of X. campestris pv. citri and (3) evaluate the role of bacteriophages in the biological control of Xanthomonads causing disease in fruit trees.

Approach: Comparative characterization of phytopathogenic xanthomonads including pathogenicity, serological characteristics, plasmid and genome DNA content, phage sensitivity, fatty acid composition, aminopeptidase activity, and isozyme activity. Develop rapid, specific, sensitive and reliable methods to detect and identify phytopathogenic prokaryote fruit tree pathogens. Develop improved inoculation and bioassay techniques to quantitatively assess pathogenicity and virulence of phytopathogenic bacteria of fruit trees and to quantitatively assess host resistance or susceptibility to infection. Determine the nature of pathogenicity of xanthomonads of fruit trees, including specific recognition phenomena and virulence factors. Identification, cloning and physical characterization of genes affecting virulence of X. c. pv. citri. Study the role of bacteriophages and antagonistic microorganisms in pathogenesis of phytopathogenic bacteria of fruit trees and development of diseases caused by these bacteria.

Progress: 88/01 to 88/12. Continued studies on the characterization of diverse Xanthomonas strains isolated from citrus including Xanthomonas campestris pv. citri (Xcc) and X. campestris (Xc) associated with citrus bacterial canker (CBC) and spot (CBS) diseases, respectively. Additional strains of Xcc from Saudi Arabia were added to the existing collection at BARC. These strains can be differentiated by serology, plasmid DNA content, genomic DNA fingerprinting, restriction fragment length polymorphism (RFLP)

and phage typing. Continued evaluation of a detached leaf bioassay further for selective isolation of Xcc and Xc variants, analyses of host-pathogen interactions and evaluation of differential host response(s) of citrus germplasm in vitro. Quantitative and qualitative evaluation of the pathogenicity of Xcc and Xc strains on detached Citrus spp. leaves by probit analyses of inoculum dose-host response in infectivity titration assays was continued. Collectively, this information can be used to differentiate Xcc and Xc variants. This information will be used by other researchers and by State and Federal action agencies, such as APHIS. Transposon mutagenesis identified pathogenicity mutants of Xcc which will be complemented from library of strain Xcb2.

Publications: 88/01 to 88/12

REISTACHER, C.N. and CIVEROLO, E.L. 1989.

Citrus bacterial canker disease of li me trees in the Maldive Islands. Plant Disease. Accepted 10/5/88.

GRAHAM, J.H., COTTWALD, T. and CIVEROLO, E.L. 1989. Population dynamics and survival of Xanthomonas campestris in soil and simulated citrus nurseries in MD and Argentina. Plant Disease. Accepted 12/21/88.

CM 10 DECIDUOUS AND SMALL FRUITS AND TREE NUTS

10.001 CRISO095488
MOLECULAR GENETIC APPROACHES FOR GERMPLASM
DEVELOPMENT OF FRUIT AND NUT CROPS

DANDEKAR A M; Pomology; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-POM-4514-H Project Type: HATCH Agency ID: CSRS Period: O1 APR 85 to 30 SEP 90

Objectives: Single genes governing resistance to disease, herbicides & environmental stresses will soon be introduced into plants of economic interest. To achieve this goal in fruit/nut crops there are many scientific hurdles to overcome: molecular characterization of variation in germplasm of fruit/nut crops; isolation of genes for resistances to disease, herbicides, environmental stresses; design suitable vector systems to deliver foreign genes; isolation of promoters sequences to allow tissue-specific control of foreign gene expression.

Approach: Development of restriction fragment length polymorphism (RFLP) studies to access variability in current cultivars of Calif. fruit/nut crops & their wild relatives; tailoring of genes responsible for drought & salinity tolerance; development of Agrobacterium based vector system to deliver foreign genes in Calif. fruit/nut crops.

Progress: 88/01 to 88/12. This research programme has forcused effort in two magor areas. 1) the development of technology for the isolation of transgenic fruit and nut plants. 2) Osmoregulation in plants. 1) We have been working with Agrobacterium in order to develop a gene transfer system. One of the factors that we have found to be important is the variation in rate of infection with different strains of Agrobacterium. We are now interested in understanding the molecular basis of this phenomenon (what genes in Agrobacterium are responsible for this variation). We are also working closely with other laboratories to develop regeneration system suitable for the regeneration of transgenic plants. In collaboration with the laboratory of Gale McGranahan we have successful in obtaining the first transgenic walnut plants. 2) We are interested in the cellular response of plants to decreasing water potential. We have carried out an analysis of the genetic control that mediates the accumulation of some organic constituents of osmotic adjustment (proline, sorbitol) by measuring their accumulation in response to decreasing water potential in Arabidopsis and walnut pollen. A single base pair change has been found in a site corresponding to a regulatory region of the first enzyme in the proline biosynthetic pathway of E. coli. This change alters feedback inhabition and is responsible for the synthesis of high levels of proline that, enable this organism to withstand decreasing water potential.

Publications: 88/01 to 88/12
 DANDEKAR, A. M. MARTIN, L. A. and MCGRANAHAN,
 G. H. (1988). Genetic transformation and
 foreign gene expression in walnut tissue.
 J. Amer. Soc. Hort. Sci. 113:945-949.

MCGRANAHAN, G. H., LESLIE, C. A. URATSU, S. L., MARTIN, L. A. and DANDEKAR, A. M. (1988). Argrobacterium-mediated transformation of walnut somatic embroyos and regeneration of transgenic plants. Bio/Technology 6:800-804.

GUPTA, P. K., DANDEKAR, A. M. and DURZAN, D. J. (1988). Somatic proembryo formation and transient expression of luciferase gene in Douglas-fir and loblolly pine protoplasts. Plant Sci. 58:85-92.

DANDEKAR, A. M. and URATSU, S. (1988). Singloe base pair change in the proline biosynthetic genes leads to osmotic stress tolerance. J. Bacteriol. 170 5943-5945.

MCGRANAHAN, G., LESLIE, C., URATSU, S., MARTIN, L. and DANDEKAR, A. (1988). Walnut tissue culture - A system for transformation. Walnut Research Reports 1987, 21-39.

MARTIN, L.A. and DANDEKAR, A.M. (1988).
Genetic transformation and foreign gene
expression in tisue of different walnut
species. Walnut Research Reports 1987,
21-39.

10.002 CRISO087835 BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA

COOKSEY D A; Plant Pathology; University of California, Riverside, **CALIFORNIA** 92521. Proj. No.: CA-R*-PPA-4259 Project Type: STATE Agency ID: SAES Period: O1 JUN 82 to 11 AUG 88

Objectives: To study mechanisms of biological control of crown gall disease and mechanisms of resistance to agrocin 84. To study interactions of bacterial pathogens with other microorganisms and develop new agents for biological control.

Approach: Investigate binding of biological control agents and mutants of these agents to plant tissue culture suspension cells. Restriction endonuclease analysisof agrocin resistant mutants. Genetic manipulations for the development of new biological control agents.

Progress: 82/06 to 88/08. A novel integrated biological/chemical control method was developed for bacterial speck disease of tomato using genetically engineered nonpathogenic, copper-resistant mutants of Pseudomonas syringae pv. tomato. The nonpathogenic mutants colonized tomato plants epiphytically and were used to exclude pathogenic strains. Since the mutants were copper resistant, they could be combined with copper bactericide treatments to provide a greater control than obtained with the mutant alone or the bactericide alone. Resistance to copper was shown to be common in strains of P. syringae pv. tomato from California. Resistance was determined by a conserved plasmid, and a physical map of the plasmid was constructed. Copper resistance genes were cloned from this plasmid and sequenced. Copper resistance was determined by four genes organized as an operon. The potential use of these genes in modifying various beneficial microbes for compatibility with copper bactericide applications is under

investigation.

Publications: 82/06 to 88/08

COOKSEY, D.A. (1988). Reduction of infection by Pseudomonas syringae pv. tomato using a nonpathogenic, copper-resistant strain combined with a copper bactericide. Phytopathology. 78:601-603.

MELLANO, M.A., and COOKSEY, D.A. (1988). Nucleotide sequence and organization of copper resistance genes from Pseudomonas syringae pv. tomato. J. Bacteriol. 170:2879-2883.

10.003* CRISO001941 GENETIC MANIPULATIONS AND GENE EXPRESSION IN WOODY PERENNIAL PLANTS.

KORBAN S S; Horticulture; 1301 West Gregory Drive, Urbana, ILLINOIS 61801. Proj. No.: ILLU-65-0325 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 91

Objectives: Combine genetic immunity to apple scab, powdery mildew, cedar-apple rust and fireblight into enhanced germplasm. Study the genetic dwarf character in Malus and incorporate it into enhanced germplasm. Investigate gene regulation and expression at the molecular level in plants and determine their behavior during plant development. Manipulate the plant genome through transformation and establish novel methods for identifying transformants. Establish cell/tissue culture protocols to induce and screen stable genetic variants.

Approach: Controlled crosses will be made among advanced apple germplasm lines to combine genes for disease resistance. Sexual hybridization will be used to study the genetic dwarf character; electrophoretic and spectrophotometric techniques will be used to characterize the gene product(s) for this trait. Techniques will be established for in vitro screening for various diseases, dwarfism, and cold hardiness using callus and cell cultures of apple, pear, peach and other woody plants. Regulatory sequences of genes encoding for LHCP II gene will be characterized and sequenced; transcription initiation site will be investigated, as well as the presence of enhancer-like elements. Chimeric genes will be constructed and used to establish transgenic plants.

Progress: 86/10 to 87/09. To identify and isolate light-harvesting chlorophyll a/b binding polypeptide (LHCP II) genes from the apple genomic library, a pea LHCP-cDNA was labeled by nick-translation and used to probe the library that was constructed earlier in our laboratory. Fifteen positive clones containing apple LHCP genes were recovered. On the basis of the restriction pattern and southern blots, an apple LHCP II gene was localized within a 14Kb insert of recombinant Charon 35 DNA. This LHCP gene has been subcloned into an M13 mp18 RF DNA vector. Clones harboring recombinant M13 DNA containing the LHCP gene were identified by color development on indicator plates and by colony hybridization. The structure of this

gene is being analyzed using Sanger's method. Screening shoot tip cultures of five apple genotypes using a hypodermic needle inoculation for fire blight infection resulted in the development of shoot necrosis in most genotypes; however, no distinction in the susceptibility of the genotypes for fire blight infection was observed. Therefore, new fire blight inoculation techniques are presently under investigation. Apple callus cultures of stem sections derived from in vitro-grown shoots were successfully established and will be induced to regenerate shoots. Seven scab resistant apple seedlings have been selected in the field based on their high fruit quality and outstanding horticultural characteristics. Two new scab resistant apple cultivars have been named and released.

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Publications: 86/10 to 87/09

CHEN. H. and KORBAN, S.S. 1987. Genetic variability, and the inheritance of resistance to cedar-apple rust in apple. Plant Pathology 36:168-174.

KORBAN, S.S., CHEN, H. and RIES, S.M. 1987. Interaction of apple cultivars with populations of cedar-apple rust. Jour. of Phytopath. 119:272-278.

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CHEN, H., ERDOS, G., KORBAN, S.S. and BEUTOW, D.E. 1987. Isolation of LHCP (II) genes from an apple genomic library. Plant Physiol. 83(4):18.

CHEN, H., KORBIN, S.S., BUETOW, D.E. and ERDOS, G. 1987. Characterization of apple LHCP genes. HortScience 22:1070.

10.004* CRISO141958 PATHOGENSIS. MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAD DISEASES OF FRUIT TREES

CIVEROLO E L; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705. Proj. No.: 1275-24000-052-00D

Project Type: INHOUSE Period: 22 AUG 86 to 21 AUG 91 Agency ID: ARS

Objectives: (1) Study pathogenecity and develop improved disease diagnostic and identification techniques for Xanthomonads; (2) identify genes affecting virulence and host recognition of X. campestris pv. citri and (3) evaluate the role of bacteriophages in the biological control of Xanthomonads causing disease in fruit trees.

Approach: Comparative characterization of phytopathogenic xanthomonads including pathogenicity, serological characteristics, plasmid and genome DNA content,phage sensitivity, fatty acid composition, aminopeptidase activity, and isozyme activity.

Develop rapid, specific, sensitive and reliable methods to detect and identify phytopathogenic prokaryote fruit tree pathogens. Develop improved inoculation and bioassay techniques to quantitatively assess pathogenicity and virulence of phytopathogenic bacteria of fruit trees and to quantitatively assess host resistance or susceptibility to infection. Determine the nature of pathogenicity of xanthomonads of fruit trees, including specific recognition phenomena and virulence factors. Identification, cloning and physical characterization of genes affecting virulence of X. c. pv. citri. Study the role of bacteriophages and antagonistic microorganisms in pathogenesis of phytopathogenic bacteria of fruit trees and development of diseases caused by these bacteria.

Progress: 88/01 to 88/12. Continued studies on the characterization of diverse Xanthomonas strains isolated from citrus including Xanthomonas campestris pv. citri (Xcc) and X. campestris (Xc) associated with citrus bacterial canker (CBC) and spot (CBS) diseases, respectively. Additional strains of Xcc from Saudi Arabia were added to the existing collection at BARC. These strains can be differentiated by serology, plasmid DNA content, genomic DNA fingerprinting, restriction fragment length polymorphism (RFLP) and phage typing. Continued evaluation of a detached leaf bioassay further for selective isolation of Xcc and Xc variants, analyses of host-pathogen interactions and evaluation of differential host response(s) of citrus germplasm in vitro. Quantitative and qualitative evaluation of the pathogenicity of Xcc and Xc strains on detached Citrus spp. leaves by probit analyses of inoculum dose-host response in infectivity titration assays was continued. Collectively, this information can be used to differentiate Xcc and Xc variants. This information will be used by other researchers and by State and Federal action agencies, such as APHIS. Transposon mutagenesis identified pathogenicity mutants of Xcc which will be complemented from library of strain Xcb2

Publications: 88/01 to 88/12

REISTACHER, C.N. and CIVEROLO, E.L. 1989.

Citrus bacterial canker disease of li me trees in the Maldive Islands. Plant Disease. Accepted 10/5/88.

GRAHAM, J.H., COTTWALD, T. and CIVEROLO, E.L. 1989. Population dynamics and survival of

GRAHAM, J.H., COTTWALD, T. and CIVEROLO, E.L 1989. Population dynamics and survival of Xanthomonas campestris in soil and simulated citrus nurseries in MD and Argentina. Plant Disease. Accepted 12/21/88.

10.005 CRISO093506 MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA DUCH.

JELENKOVIC G; CHIN C; HOLLAND M; Horticulture & Forestry; Rutgers University Po Box 231, New Brunswick, **NEW JERSEY** 08903.

Proj. No.: NJ12110 Project Type: HATCH Agency ID: CSRS Period: 29 AUG 84 to 30 SEP 89

Objectives: Determine the number of rRNA genes per genome in a selected number of genotypes of the octoploid strawberry. Establish a development and growth profile of the seedlings obtained by selfing and intercrossing of the genotypes having dramatically different number of rRNA genes. Determine the number of rRNA genes in representative samples of seedlings of various phenotypic classes formed by the developmental and growth characteristics in the progenies. Produce a 'genome library' for the cultivated octoploid strawberry. Clone an 'entire gene' of rRNA genes in one of the plasmids.

Approach: DNA and RNA will be isolated, purified and labelled with Pusing r-P-ATP in a T(4) polynucleotide Rinase reaction. Then the multiplicity of rRNA genes will be assayed by filter hybridization methods developed by Gillespie and Spiegelman. Whether there is correlation of the number of rRNA genes of various genotypes and their growth and developmental characteristics will be examined. The rRNA gene will be clones using the method of Gerlack and Bedbrook.

Progress: 88/01 to 88/12. 1 of the isolated genomic clone lamda 5511, of alcohol dehvdrogenase (Adh) from the cultivated octoploid strawberry (genotype NJ8343-6) has been characterized by restriction & sequence analysis. The original clone of 23kb has been subcloned & a fragment of 5.5 kb was identified by heterologous hybridization (maize probe) to contain the Adh sequence. This fragment has been further subcloned & a series of unilateral, overlapping deletions has been produced. The latter were used for sequencing by double stranded dideoxy sequencing method. In toto 3,210 nucleotide pairs (np) were sequenced, including entire coding region & 500 np upstream & 400 np downstream of the coding region. Based on homology w/ maize Adh gene, the coding region of strawberry gene consist of 10 exon & 9 introns. The 9 introns begin w/ GT & terminate w/ AG, & are AT reach (65-72%). The exons are of the same lenght as in maize except the 1st one which is longer for 1 codon, & monomeric form of strawberry Adh should consist of 380 amino acid. The coding region of the strawberry Adh gene is 74-76% homologous to Adh in arabidopsis, barley maize & peas; homology at proteins level is 81-85%. The regulatory sequences TATA & AGGA boxes were tentatively located at 363~3709 & 343-352 respectively (the 1st triplet of the 1st exon being at 520). The core consensus sequence for anaerobic induction of Adh (TGGTTT) in strawberry consists of 4 tandem repeats at position of 223.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

10.006 CRISO087911
ALLOZYME AND DNA POLYMORPHISMS AS GENETIC
MARKERS IN CROPS

WEEDEN N F; Horticultural Science; N Y Agriculture Expt Station, Geneva, $\mbox{NEW YORK}$ 14456.

Proj. No.: NYG632491 Project Type: HATCH

Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: To establish linkage maps using idozyme loci and DNA restriction fragment length polymorphisms in several crop species including garden pea, lentil, common bean, and apple. These biochemically-defined loci will be used to select markers for commercially important traits, identify instances of synteny within the Leguminosae, and to isolate DNA fragments containing other genes of interest.

Approach: Survey the germplasm in beans and apple for new variation, using this variation in crosses to determine the mode of inheritance and linkage relationships and identification of appropriate marker loci. We will pursue the use of cloned DNA fragments to identify restriction=fragment length polymorphisms and use these as additional genetic markers.

Progress: 88/01 to 88/12. Progress continues to be made in the generation of chromosomal linkage maps for garden pea, lentil, apple and grape. In pea, an isozyme locus (ADH-1) was shown to be an excellent marker for En, the locus controlling resistance to pea enation mosaic virus. The location of several previously unmapped isozyme and DNA polymorphisms were determined as well as those of Sn, a gene controlling flowering, and 13 genes involved in nodule formation and nitrogen fixation in pea. A comparison of the preliminary linkage maps for pea and lentil identified several linkage groups in common, suggesting that the parallel mapping of the pea and lentil genomes should work synergistically to increase the rate of progress in either species. Isozyme and DNA markers also have been very useful for genetic studies in apple and grape. In apple, a rudimentary linkage map was developed using isozyme loci and DNA restriction fragment length polymorphisms. We published a genetic analysis of allozyme polymorphisms in grape and demonstrated that techniques initially developed for apple genetics can be applied satisfactorily to similar analyses in grapes.

Publications: 88/01 to 88/12

- WEEDEN, N. F. 1988. A suggestion for the nomenclature of isozyme loci. Pisum Newsletter 20:44-45.
- WEEDEN, N. F. 1988. Polymorphic isozyme loci identified in Pisum. Pisum Newsletter 20:46-48.
- WEEDEN, N. F. and HAGENS, D. 1988. Linkage between Tip-p and Le. Pisum Newsletter 20:42-43.
- WEEDEN, N. F., KNEEN, B. E., and MURFET, I. C. 1988. Mapping of Sn to chromosome 2. Pisum Newsletter 20:49-51.
- WEEDEN, N. F., KNEEN, B. E., and LARUE, T. A. 1988. Mapping genes in Pisum which affect the host plant's ability to form nodules. Genome 30:S287.

10.007 CRISO133699 MOLECULAR GENETICS OF THE COCOA TREE THEOBROMA CACAO

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FRITZ P J; Food Science; Pennsylvania State University, University Park, **PENNSYLVANIA** 16802.

Proj. No.: PENO2973 Project Type: HATCH Agency ID: CSRS Period: O1 JAN 88 to 30 JUN 92

Objectives: To use the tools of modern Plant Science, in particular the ability to isolate and transfer genes, to genetically engineer a better cocoa tree.

Approach: Perfect methods for growing cocoa cells and explants in culture, including the ability to micropropagate and regenerate whole plants. Perfect methods for transferring genes, either homologous or heterologous, into cocoa cells. Identify genes needed for improving the plant, including, for example, those needed for disease resistance.

Progress: 88/01 to 88/12. 1. A vegetative propagation system for Theobroma Caca capable of yielding as manay as 5,000 propagules annually was developed. The system is based on axillary bud proliferation and is designed to produce trees from the more desirable orthotrpoic shoots. 2. Cocoa chloroplast DNA was shown to have a genome size of about 100 kilobase pairs. Twenty-five percent of the chloroplast genome was cloned. 3. It was established by meiotic chromosome pairing, numbers of nucleoli per nucleus, and in situ hybridization that cocoa is a diploid taxom. 4. Restriction fragment length polymorphism (RPLP) technology was developed for cocoa 5. Progress in developing a cocoa transformation system using Agrobacterium tumefaciens was made. 6. Progress on characterizing cocoa genomic DNA was made. 7. Progress on isolating the cocoa seed glycerol - 3 - phosphate acyl transferase gene was made.

Publications: 88/01 to 88/12

- FRITZ, P. J. 1988. Theobroma Cacao DNA as a marker for Plant Breeding. Proc. 1st Pan American Development Foundation Cocoa Forum.
- CHUNG, D. K. 1988. Theobroma cacao Chloroplast DNA: Isolation and Characterization. (Thesis) Penn State University.
- MIRAZON, M. 1988. Theobroma cacao: Genetic Analysis Using Restriction Fragment Length Polymorphisms. (Thesis). Penn State University.
- FLYNN, W., FRITZ, P. J. Invention disclosure A Micropropagation System for Theobroma Cacao.

10.008 CRISO099027 GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION

KRUL W R; Plant Science; University of Rhode Island, Kingston, **RHODE ISLAND** 02881.

Proj. No.: RIR-8600544 Project Type: CRG0 Agency ID: CRG0 Period: 01 SEP 86 to 31 AUG 89

Objectives: Proj. 8600544. Confirm mode of inheritance and function of resistance genes in Vitis and Nicotiana to wide and limited host range strains of Agrobacterium tumefaciens; determine which pathogen genes are required for infectivity, elicitation of the hypersensitive response; and the modulation of pathogen and host genes by phytohormones.

Approach: Genetics of plant resistance will be determined by conventional breeding methods coupled to screens with wild type and genetically modified pathogen races with or without phytohorome pretreatment.

Progress: 87/10 to 88/09. The interactions of virulence (vir) and tumor inducing (T-SNA) genes of the plant pathogen Agrobacterium tumefaciens and Vitis and Nicotiana species and interspecific hybrids were examined. Incompatible reactions to the wide host range (WHR) stains of the bacterium were conditioned by both dominant and recessive plant genes. The recessive plant gene(s) regulates a hypersensitive reaction in response to wide host range strains. A restriction map of the limited host range (LHR) vir region has been completed and strains containing lacZ gene from E. coli have been inserted into all vir loci. Some lacZ mutants were marker exchanged into the limited host range bacterium and will be tested for infectivity on grapevine. Inducers of LHR virulence genes from Vitis, Lycopersicon and Nicotiana species are not homologous to the wide host range inducer acetosyringone or to simple phenolic compounds. Compounds that induce WHR strains do not induce LHR strains. All LHR inducers obtained thus far have molecular weights 1000 and induce both LHR and WHR vir loci. Vir gene repressors were obtained from resistant species of Nicotiana and the non host maize. Genetic analysis of a maize mutant (repressor minus and vir inducer(s) plus) shows that a single dominant gene modulates repressor content. Inducer and repressor compounds may modulate infectivity and host selectivity of the pathogen.

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Publications: 87/10 to 88/09 NO PUBLICATIONS REPORTED THIS PERIOD.

10.009 CRISO137399
RFLP AND MOLECULAR ANALYSIS OF ROOT KNOT
NEMATODES, NEMATODE INFECTED PLANTS AND PEACHES

ABBOTT A G; BILLARD R; LEWIS S A; Biological Sciences; Clemson University, Clemson, **SOUTH CAROLINA** 29634.

Proj. No.: SC01310 Project Type: HATCH Agency ID: CSRS Period: 01 FEB 89 to 31 DEC 92

Objectives: Generate DNA clones for the construction of RFLP (restriction fragment length polymorphism) maps in peach and for race identification in nematodes; cDNA clones of nematode infected plant tissue will be used to search for plant genes induced during giant cell formation.

Approach: Peach RFLP maps will be constructed using random genomic clones in pUC8 and polymorphisms detected in hybrid peach X almond

crosses. Random clones of the nematode species M. ingognita will be screened for polymorphism in biotypes 1, 2, 3 and M. areneria biotypes 1 and 2. Cascade hybridization will identify clones unique to M. incognita infection of soybean tissue. These will be studied in the hypersensitive response.

CM 11 POTATOES

11.001 CRISO093496 EXPLORATORY RESEARCH ON THE CYTOGENETICS OF SOLANACEOUS CROPS

QUIROS C F; Vegetable Crops; University of California, Davis, **CALIFORNIA** 95616.

Proj. No.: CA-D*-VCR-4452-H Project Type: HATCH Agency ID: CSRS Period: O1 OCT 88 to 30 SEP 93

Objectives: Develop markers to study interploid crosses. Evaluate variability and erosion in Andean primitive cultivars. Determine interspecific hybridization and introgression of weedy and cultivated potatoes in the Andes.

Approach: Intercross 2n pollen (FDR) and 2n egg (SDR) producing diploid strains heterozygous for isozyme loci. Determine the diploid/tetraploid ratio for each progeny. Test for expected SDR and FDR ratios in the resulting tetraploids. Calculate transmission of parental genotypes. Quantify with markers the variability of Andean cultivars. Determine intra- and interspecific hybridization of cultivated and weedy species in Andean experimental plots. Ascertain level of interploidy hybridization and its possible association to the origin of polyploid species.

Progress: 88/01 to 88/12. Isozyme markers were used to survey the genetic variability of non-bitter potatoes in subsistence fields of Andean farmers at 3600 to 3800 m above sea level. Sixty-seven percent of the varieties were tetraploid corresponding to the species Solanum tuberosum ssp. andigena, 14% were triploids, probably corresponding to S. xchaucha, and 13% were diploids corresponding to the species S. phureja, S. stenotomum and S. goniocalyx. A high degree of correspondence between farmer identification and electrophoretic phenotypes was observed. The amount of variability observed in the sample of Andean potatoes was superior to that present in North American and European varieties. This finding supports the notion that a substantial amount of yet exploited variability remains in Andean potato populations. In a separate study, a high level of interspecific hybridization (95%) was observed in Andean fields between the diploid cultivated species S. stenotomum and the diploid weed S. sparsipilum. This high level of hybridization is likely to play an important role in generating new variability thus accounting for the large number of varieties reported in the Andean Region.

Publications: 88/01 to 88/12

DOUCHES, D.S. and QUIROS, C.F. (1987).

Genetic recombination in a diploid synaptic mutant and a Solanum tuberosum x S. chacoense diploid hybrid. Heredity 60:183-191.

RABINOWITZ, D., LINDER, C.R., DOUCHES, D.S. and QUIROS, C.F. (1988). Determining natural hybridization between S. sparsipilum and S. stenotomum in the Andes. Amer. Potato J. 65:496.

11.002 CRISO131676 CONTROL OF GENE EXPRESSION IN POTATO

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ROXBY R; Biochemistry; University of Maine, Orono, MAINE 04469.

Proj. No.: MEO8406 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 87 to 30 SEP 92

Objectives: Genes differentially expressed in a potato tissue culture system induced by cytokinin to form tubers will be isolated and the expression characteristics of them analyzed. The tuberization response under natural conditions will be compared to that in culture. In a separate project, the structure of the potato chitinase gene will be investigated and the role of upstream regulatory sequences assessed.

Approach: Differential screening of cDNA libraries with probes isolated from induced and uninduced tissue will be used to isolate differentially expressed clones. Expression will be measured using blotting techniques. Upstream regulatory sequences of the chitinase gene, systematically mutated with deletions, will be fused to a reporter gene. These constructs will be used to generate transformed potato plants in which the expression of the transgenes will be monitored.

Progress: 87/10 to 88/09. A series of six chitinase cDNA clones has been isolated from potato and characterized by restriction mapping and partial sequencing. Two different classes of clones can be identified by restriction mapping. Sequencing different members of the same restriction map class reveals further differences among them that suggests that the mRNAs they represent genes. The detection of multiple cDNA clones is in accord with the observation of multiple bands hybridizing to chitinase probes in Southern blots and the isolation in other laboratories of multiple proteins having chitinase activity. A gene construction designed to produce large quantities of an RNA complementary to the mRNA produced by one of the sequenced potato genes has been prepared by connecting one of the cDNA clones to the Cauliflower mosaic virus promoter. The object of this experiment is to determine whether chitinase gene expression can be measurably reduced in transformants into which this construct has been introduced.

Publications: 87/10 to 88/09
 LAFLAMME, D., and ROXBY, R. (1988). Isolation
 and characterization of potato genes
 hybridizing to a bean chitinase probe.
 Jour. Cellular Biochem. Supplement 12C,
 p216.

11.003 CRISOO96017 ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES

ROXBY R W; Biochemistry; University of Maine,

Orono, **MAINE** 04469.

Proj. No.: ME35159 Project Type: CRG0 Agency ID: CRG0 Period: O1 JUL 85 to 30 JUN 88

Objectives: Proj 8500319. Cloning and characterization of genes expressed in response to tuberization stimuli in potato.

Approach: Cloning, as cDNA, of mRNA differentially expressed upon exposure of potato plants or tissues to tuberization stimuli which may be either environmental (short day, cool temperature) or hormonal (high cytokinin levels in culture). Genomic sequences corresponding to the mRNA clones will be isolated from a genomic library, structures determined and 5' flanking sequences analyzed in detail, particularly with respect to the ability of those sequences to direct tissue specific expression of tuber genes.

Progress: 87/10 to 88/09. Differential screening of potato shoots induced to form tubers against similarly treated control shoots has lead to the isolation of a number of cDNA clones that may represent genes involved in early stages of tuberization. The screening was done with complex probes, a relatively insensitive way to search for such genes. To improve the methodology, directionally cloned cDNA libraries of induced and control tissue have been made in vectors from which plus and minus sense RNA can be transcribed from the cloned insert. Subtractive hybridization of transcribed RNA populations will be carried out and the sequences unique to the induced population fractionated by HAP chromatography, using procedures we have developed. This will provide material for probing or cloning which will enhance the sensitivity of the differential detection methods.

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Publications: 87/10 to 88/09 COOK, L. 1988. Cloning of mRNA differentially expressed upon hormonal induction of tuber formation in potato. M.S. Thesis, University of Maine.

11.004 CRISO098604 GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X

TAVANTZIS S; Botany & Plant Pathology; University of Maine, Orono, MAINE 04469. Proj. No.: ME35164 Project Type: SPECIAL GRANT Agency ID: CSRS Period: 15 APR 86 to 31 OCT 87

Objectives: The overall objective of the project is to transfer the single, dominant gene Rx, conferring extreme resistance to potato virus X (PVX), to commercial potato varieties susceptible to PVX. The specific objectives are to: Study the nature of extreme resistance (or operational immunity) attributed to gene Rx; isolate, clone and sequence gene Rx; transform protoplasts or leaf disks from PVX-susceptible plants with the Rx gene.

Approach: Research conducted in my lab has shown that Rx-conferred resistance is expressed in protoplasts from Rx plants and appears to be resistant to virus replication. Experiments will be carried out at the protoplast level to determine the phase of the virus replication cycle with which Rx-conferred resistance interferes, and how this is brought about. Gene

Rx will be isolated by identifying protein(s) that are specifically associated with Rx genotypes; differential hybridization aimed at the comparison of mRNA populations between Rx and Rx plants; transposon mutagenesis, using transposable elements from snapdragon, which is taxonomically related to potato.

Progress: 87/10 to 88/09. The diploid line USW 2225, which carries gene Rx conferring extreme resistance to PVX, was transformed with the maize transposable En-1 via Agrobacterium tumefasciens infection. The resulting potato clone PEn was shown to express En-1 functions as determined by Northern blot hybridization. Transposition of En-1 in PEn was demonstrated by Southern blot hybridization and most rigorously by sequencing of excision and transposition products. An increasing number of En-1 specific bands is seen with prolonging times of exposure of the Southern blots, which suggests that transposition is not only an ongoing process during somatic development of the PEn plants but that it occurs with a rather high frequency. In the two cases tested, transposition has occurred into unique potato DNA as documented by Southern blot hybridization. These results clearly demonstrate that the En-1 element of maize can be used for transposon-mediated gene tagging in potato. Currently we are building up the proper heterologous material for tagging of the virus X resistance gene (Rx), which is present n our transgenic potato clone PEn.

Publications: 87/10 to 88/09
FREY, M., TAVANTZIS, S.M. and SAEDLER, H.
 1988. The maize En-1/spm element transposes
 in potato. The European Molecular Biology
 (EMBO) Journal (In Press).

11.005 0097014 MOLECULAR GENETICS OF EXTREME RESISTANCE TO POTATO VIRUS X

TAVANTZIS S; Botany & Plant Pathology; University of Maine, Orono, MAINE 04469. Proj. No.: MEO8451 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 89 to 30 SEP 94

Objectives: The ultimate objective of this project is to engineer commercial potato varieties with the single dominant gene Rx which confers extreme resistance to infection by potato virus X (PVX). The specific objectives of the proposed research are to: elucidate the mechanism of resistance conferred by the Rx gene; isolate, clone, and sequence gene Rx, and; introduce gene Rx to commecial potato cultivars.

Approach: The expression of gene Rx, as determined by the extent of PVX replication, will be studied in intact plants and protoplasts carrying the gene. The degree of resistance to virus replication and translocation conferred by gene Rx as well as tissue specificity, light inducibility, and temperature sensitivity will be monitored by working with intact Rx e.g., PVX-resistant) potato plants. To understand the mechanism of the Rx-mediated resistance, protoplasts from Rx

and Rx potato genotypes will be inoculated with PVX and the ratios of the different components of the PVX replication cycle will be compared to determine with which step of the PVX replication cycle the Rx gene interferes. Gene Rx will be located by transposon mutagenesis or RFLP mapping techniques and positively identified by Agrobacterium- mediated transformation of Rx potato leaf disks giving rise to PVX- resistant transgenic plants. Cloning and sequencing of gene Rx and potato transformation will be carried out using standard procedures.

Progress: 87/10 to 88/09. The objective of this project is to isolate the single, dominant gene Rx which confers extreme resistance to potato virus X (PVX) in potato. The gene will be used to incorporate effective resistance to PVX into commercial potato varieties and study how resistance is brought about. In collaboration with two other laboratories, three approaches are followed to achieve the above objective. In a joint project with scientists of the Max Planck Institute, Cologne, FRG, we have introduced the maize transposon En-1/Spm to a potato line which possesses gene Rx. We have shown that the maize element is active in potato i.e. it transposes ("jumps") from one position in the genome to another with a high frequency. So, it is possible now to locate gene Rx using the techniques known as transposon mutagenesis or transposon tagging. In other collaborative effort with scientists at Cornell University, we are trying to map gene Rx on the potato genome using a technique known as gene mapping by restriction fragment length polymorphism (RFLP). Using this approach gene Rx will be mapped with in a chromosomal fragment of several kilobase pairs. Portions of the above segment will be subcloned and introduced into a PVX-susceptible potato line to see which portion is associated with conversion to PVX resistance. Subsequent location of gene Rx should be accomplished without major problems.

Publications: 87/10 to 88/09
FREY, M., TAVANTZIS, S.M. and SAEDLER, H.
 1988. The maize En-1/Spm element transposes
 in potato. The European Molecular Biology
 (EMBD) Journal (In Press).

11.006 CRISOO34187 MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI

COLLMER A; Botany; University of Maryland,
College Park, MARYLAND 20742.
Proj. No.: MD-8401366 Project Type: CRGD
Agency ID: CRGD Period: 01 JUL 84 to 30 JUN 86

Objectives: Determine the genetic basis for the production of multiple isozymes of pectate lyase (PL) in Erwinia chrysanthemi CUCPB 1237. Evaluate the functional difference between PL isozymes.

Approach: Clone all of the PL isozyme structural genes in plasmid vector pBR322. Restriction map the cloned fragments. Use polyacrylamide gel electrophoresis in

conjunction with a high resolution activity stain to determine the actual complexity of the E. chrysanthemi PL isozyme profile and to analyze the PL isozymes produced by various recombinant plasmids. Prepare highly purified PL isozymes using subcloned genes, expression vectors and affinity chromatography. Biochemically characterize PL isozymes with respect to interactions with host tissue preparations. Use site-directed mutagenesis and complementation techniques with subcloned isozyme genes to evaluate the contribution of each isozyme to the catabolic, plant parasitic and pathogenic capacities of E. chrysanthemi.

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Progress: 84/07 to 86/06. phytopathogenic enterobacterium Erwinia chrysanthemi contains pel genes encoding several different isozymes of the plant-tissue-disintegrating enzyme pectate lyase (PL). The obstacles to genetic analysis of structural genes when a phenotype is conferred by multiple isozymes was overcome by manipulating the genes after molecular cloning. The pelC gene encoding isozyme PLc was mutagenized by a three-step procedure involving (i) insertional inactivation of the cloned gene by ligation of a kan-containing BamHI fragment from pUC4K with a partial Sau3A digest of E. chrysanthemi strain CUCPB 1237 pelC DNA in pBR322; (ii) mobilization of the pBR322 derivative from Escherichia coli to E. chrysanthemi; and (iii) exchange recombination of the pelC::kan mutation into the E. chrysanthemi chromosome by selection for kanamycin resistance in transconjugants cultured in phosphate-deficient medium (which renders pBR322 unstable). The resulting E. chrysanthemi mutant was Kanr, Amps, lacked pBR322 sequences, and was deficient in only one of the four major PL isozymes, PLc, as determined by activity-stained isoelectric-focusing polyacrylamide gels. A similar procedure was also used to inactivate pelB and pelE (in strain EC16 which naturally fails to make PLd). The mutants were analyzed for their ability to grow and cause maceration in injected whole potato tubers.

Publications: 84/07 to 86/06

CDLLMER, A. and KEEN, N.T. 1986. The role of pectic enzymes in plant pathogenesis. Annu. Rev. Phytopathol. 24:383-409.

CDLLMER, A., SCHDEDEL, C., RDEDER, D.L., RIED, J.L. and RISSLER, J.F. 1985.

Molecular cloning in Escherichia coli of Erwinia Chrysanthemi genes encoding

multiple forms of pectate lyase. J. Bacteriol. 161:913-920.
ROEDER, D.L. and CDLLMER, A. 1985.
Marker-exchange mutagenesis of a pectate lyase isozyme gene in Erwinia chrysanthemi.

J. Bacteriol. 164:51-56.

RDEDER, D.L. and CDLLMER, A. 1986.

Marker-exchange mutagenesis of the pelB gene in Erwinia chrysanthemi CUCPB 1237. In Proceedings of the VI International Conference on Plant Pathogenic Bacteria, June 2-6, 1985.

RIED, J.L. and CDLLMER, A. 1986. A kan, sac cartridge for generating directed, unmarked mutations in Gram-negative bacteria. Phytopathology (abstr. in press).

11.007 CRISOO98873 DEVELOPMENT OF A DNA HYBRIDIZATION ASSAY FOR DETECTION OF THE RING ROT PATHOGEN

OLESON A E; GUDMESTAD N C; MOGEN B D; Biochemistry; North Dakota State University, Fargo, NORTH DAKOTA 58105.

Proj. No.: ND05021 Project Type: SPECIAL GRANT Agency ID: CSRS Period: 01 JUL 86 to 30 SEP 88

Objectives: Development of a recombinant DNA probe specific for Corynebacterium sepedonicum, the causal agent of bacterial ring rot of potatoes. Development of a hybridization assay for the ring rot pathogen and assessment of the sensitivity and specificity of the method in reconstructed and natural samples.

Approach: The specific probe will be obtained by preparing a library of cloned genomic fragments of C. sepedonicum and screening this library by the plus/minus method using nick-translated DNA from C. sepedoncium and other coryneform species. The hybridization assay will utilize standard dot-blot methodologies, and densitometry will be used to quantitate signal intensities. The effect of commonly encountered sample matrices on the hybridization procedure will be evaluated.

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Progress: 86/07 to 88/09. The genome of Clavibacter michiganense subsp. sepedonicum, the potato bacterial ring rot pathogen, contains a repeated sequence (RS). Two copies of the RS are present on the single indigenous plasmid, pCS1, found in this organism, and ca. 200 copies are present on the bacterial chromosome. A 1900-bp plasmid fragment containing the RS has been cloned and sequenced. Oligonucleotides (20 bases each) were synthesized from regions throughout the sequenced fragment and used to probe Southern blots of Sma-cut chromosomal DNA. This study indicated that the RS is located near the center of the sequenced fragment. Computer analysis of the sequence indicates that the RS is an IS-like element. The RS is a 1092-bp structure that contains a terminal inverted repeat of 32 bp which flanks a gene coding for a 36-kDa protein. A BamHI restriction fragment of the sequenced portion of the plasmid was subcloned into a transcription vector. Radiolabeled run-off transcripts were prepared for use as a probe of the C. m. sepedonicum RS. Tests of the specificity of this probe with chromosomal DNA of many different bacteria revealed that only C. m. insidiosum, the alfalfa wilt pathogen cross reacted with the probe. Melting studies of probe-target DNA complexes revealed that the RS from C. m. insidiosum is only partially homologous with the RS from C. m. sepedonicum. High stringency hybridization conditions permit selective detection of the ring rot pathogen.

Publications: 86/07 to 88/09

MOGEN, B.; OLESON, A.; SPARKS, R.; GUDMESTAD, N.; and SECOR, G. 1988.

Distribution and partial characterization of pCS1, a highly conserved plasmid present in Clavibacter michiganense subsp. sepedonicum. Phyto. 78:1381-1386.

OLESON, A. 1988. Recombinant probes for plant pathogenic microbes. In: 21st Century Prospects for Biotechnology in Agriculture and Environment. Agrogen, Slusovice, Czechoslovakia. (in press).

11.008 CRISO135894 BASIC AND APPLIED MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN

OLESON A E; SPARKS R B; GUDMESTAD N C; Biochemistry; North Dakota State University, Fargo, NORTH DAKOTA 58105.

Proj. No.: ND01232 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: (1) Characterize the structure and function of plasmid pCS1 of Clavibacter michiganense subsp. sepedonicum. (2) Develop a cloning vector and transformation system for this plant pathogen. (3) Develop a dot-blot DNA hybridization method for detection of the ring rot pathogen. (4) Develop a method for differentiation of pathogen strains based on RFLP analysis.

Approach: Transcripts and translational products of pCS1 will be analyzed, and possible biological activities encoded by the plasmid will be examined. Recombinant constructs based on plasmids from Gram-positive organisms will be generated for use as a cloning vector. Transformation will be accomplished by a method based on polyethylene glycol-mediated uptake of DNA by lysozyme-penicillin protoplasts. Detection and strain differentiation of the pathogen will utilize a recombinant probe containing a high-copy-number IS-like element cloned from a DNA fragment of the C. m. sepedonicum genome.

11.009 CRISO142982 RECOMBINANT DNA APPROACH TO DETECTION AND STRAIN DIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN

GOTH R W; OLESON A E; GUDMESTAD N C; Biochemistry & Molecular Bio; North Dakota State University, Fargo, NORTH DAKOTA 58105. Proj. No.: 0500-21010-016-15S

Project Type: COOPERATIVE AGREE. Agency ID: ARS Period: O1 JUL 88 to 30 JUN 90

Objectives: 1) Development of a dot-blot DNA hybridization method for detection of the ring rot pathogen. 2) Development of a method for differentiation of pathogen strains based on restriction fragment length polymorphism (RFLP) analysis.

Approach: A cloned repeated sequence from Corynebacterium sepedonicum will be used as a probe in development of a dot hybridization method for pathogen detection. The relative signal strengths produced by all available strains of the pathogen will be tested, and the sensitivity of the method will be compared with current methods. Other organisms will be tested

for false positive responses, and sample matrix effects will be examined. The cloned sequence will also be used as a probe for a RFLP-based method for strain differentiation. The patterns obtained with all available strains of the pathogen will be examined. The stability of the observed restriction patterns will be tested by cycling the pathogen through the natural infection process in susceptible plants and through nutrient media.

Progress: 88/07 to 88/12. Efforts during this period on dot hybridization method for pathogen detection focused on enhancing the specificity of the cloned molecular probe for the potato ring rot pathogen. Effect of varying stringency of hybridization washes has been investigated to determine the re- latedness of the target sequence of Clavibacter michiganense sepedonicum and C.m. insidiosum the organism that shows the greatest cross reaction with the C.m. sepedonicum repeated sequence (RS) riboprobe . The baked membrane containing genomic DNA from both organisms was hybridized with the riboprobe and then washed successively with standard wash solution (0.1% SSCP containing 1% SDS) at temperatures from 50 to 85 C. Melting temperatures of the bound probe were ascertained from a plot of the residual bound radiolabel as a function of the wash temperature. The Tm of the complex with C.m. sepedonicum was found to be 81 C, whereas that with C.m. insidiosum was 71 C. This result indicates that the repeated sequence in the alfalfa bacterial wilt pathogen is similar but not identical to that found in the potato bacterial ring rot pathogen. Differentiation of ring rot pathogen strains by restriction fragment polymorphism analysis has been directed at examination of the effect of extended growth of the pathogen in culture at standard temperature ((20 C) and at two more stressful temperatures (10 C and 30 C). This research will benefit State potato seed certification agencies, growers, and scientists.

Publications: 88/07 to 88/12
ND PUBLICATIONS REPDRTED THIS PERIDD.

11.010 CRISO098603 ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT

GUDMESTAD N C; DLESDN A E; BDE A A; Plant Pathology; North Dakota State University, Fargo, NORTH DAKOTA 58105.

Proj. No.: NDO5124 Project Type: SPECIAL GRANT Agency ID: CSRS Period: O1 JUL 86 to 30 SEP 88

Objectives: Molecular approaches to ring rot detection; development of a virulent isolate of C. sepedonicum containing a selectable antibiotic resistance gene. Utilization of cloned fragments of the C. sepedonicum chromosome and its plasmid pCsl as specific probes for restriction - fragment length polymorphism (RFLP) analysis of the genomic diversity of the C. sepedonium population. Development of improved potato varieties; to evaluate the existing germplasm of Solanum tuberosum L. and other tuber-bearing solanums

for characteristics related to fresh and processing quality, storage characteristics, yield, resistance to hollow heart and resistance to pests. To develop breeding lines (germplasm) that carry genes for specific quality characteristics using conventional breeding and biotechnology methods.

Approach: Ring rot detection; transformation of C. sep. isolates with plasmids encoding antibiotic genes from other Corynebacterium sp. using polyethylene glycol-mediated DNA uptake. RFLP analysis using radiolabeled cloned genes of C. sep. as probes. Potato variety development. Development of improved potato varieties will be accomplished by screening germplasm of S. tuberosum and other tuber-bearing species for the above characteristics. Specific genes will be incorporated using convention breeding and biotechnology approaches.

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Progress: 87/10 to 88/09. Potato ring rot research. Restriction fragment length polymorphism (RFLP) analysis was developed in order to aid in the characterization of naturally occurring strains of Corynebacterium sepedonicum. RFLP analysis is performed on both plasmid and chromosomal DNA and is useful in separating strains in which the plasmid DNA is either autonomous or integrated into the chromosome. RFLP patterns were found to be extremely stable even after repeated subculturing. A 1.5 kb segment of plasmid DNA from a repeated sequence is being tested for its usefulness in developing a DNA hybridization probe that can be used to detect the ring rot bacterium in plant tissue. The probe is sensitive and highly specific for C. sepedonium. Varietal improvement. Progress was made in analyzing the inheritability of low reducing sugar accumulation, calcium uptake and utilization and storage performance. Individual clones were selected that had reducing sugar values lower than the lowest parent. Calcium uptake is being analyzed by atomic emission spectroscopy and is in progress.

Publications: 87/10 to 88/09

MDGEN, B.D., OLESON, A.E., SPARKS, R.B.,
GUDMESTAD, N.C. and SECDR, G.A. 1988.
Distribution and partial characterization of
pcs1, a highly conserved plasmid present in
C. michiganese subsp. sepedonicum.
Phytopath. 78:1381-1386.

11.011 CRISO131999 INTEGRATED APPROACH FOR POTATO BACTERIAL DISEASE CONTROL AND VARIETY IMPROVEMENT

GUDMESTAD N C; DLESDN A E; BDE A A; Plant Pathology; North Dakota State University, Fargo, NORTH DAKOTA 58105.

Proj. No.: NDO5126 Project Type: SPECIAL GRANT Agency ID: CSRS Period: O1 JUL 87 to 30 DEC 89

Objectives: Potato Bacterial Disease Control. Utilization of cloned fragments of the C. sppedonicum chromosome and its plasmid pCs1 as specific probes for restriction-fragment length polymorphism analysis of the genomic diversity of the C. sepedonicum population. Development

of a virulent isolate of C. sepedonicum containing a selectable antibiotic resistance gene. Development of a system to differentiate between strains of C. sepedonicum that originate from sugar beet and potato using analysis of cellular fatty acids. Development of an Erwinia-caused disease management scheme emphasizing factors that are under the potential control of the grower. Potato Germplasm and Cultivar Improvement. To evaluate existing germplasm of Solanum tuberosum and other tuber-bearing solanums for characteristics related to fresh and processing quality, storage characteristics, yield, resistance to hollow heart and resistance to pests.

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Approach: Construct genomic library of C. sepedonicum using restriction enzyme digests. Use C. glutamicum as plasmid vector. Fatty acid composition analysis using gas-liquid chromatography. Evaluate seed handling practices and their effects. Evaluate diploid x tetraploid crosses for desirable characteristics and utilize tissue culture techniques to accelerate screening.

Progress: 87/10 to 88/09. Potato Bacterial Disease Control. The plasmid of Corynebacterium sepedonicum contains a highly conserved repeated sequence that exists as 200-400 copies per genome in all of the strains tested. When this repeated sequence was tested against other plant pathogenic coryneform bacteria, no cross-hybridization was detected. This probe is being further developed for the detection of ring rot bacteria in potato. Fatty acid analysis of cellular components has demonstrated the presence of five major fatty acids in C. sepedonicum. Qualitative and quantitative differences in fatty acid content has permitted the separation of ring rot bacteria from other plant pathogenic and nonpathogenic coryneform bacteria. Variety Improvement. A genetic study of "cold chipping" ability in potatoes found the trait to be under recessive gene control, with epistatic effects. A new study is evaluating the relationship between respiration level and sugar accumulation. Breeding families continue to be screened for "cold chipping" ability, and valuable materials directed into the varietal breeding program. Calcium uptake and its relationship to tuber defects and storage performance continues to be studied. Breeding populations ustilizing S. cardiophyllum and S. commersonii are being developed. Several populations utilizing S. commersonii have been field planted and will be evaluated for tuber quality this winter.

Publications: 87/10 to 88/09 GUDMESTAD, N. C., HENNINGSON, P. J. and BUGBEE, W. M. 1988. Cellular fatty acid comparison of strains of Corynebacterium michiganense subsp. sepedonicum from potato and sugar beet. Can. J. Microbiol. 34:716-722.

11.012 CYTOPLASMIC FACTORS OF THE POTATO

CRISO096337

GRUN P; COLE R; Horticulture; Pennsylvania State University, University Park, **PENNSYLVANIA** 16802.

Proj. No.: PENO2829 Project Type: HATCH Agency ID: CSRS Period: O1 SEP 85 to 30 JUN 90

Objectives: Determine stability of cytoplasms of sterility resistant backcross lines (BC1's); survey quality characters of BC1's; analyze evolution of potato mitochondrial DNA (mtDNA), mtDNA stability, and relation of mtDNA to cytoplasmic sterilities.

Approach: Produce reciprocal progenies from crosses between BC1's and tester plants having known cytoplasm-sensitive genes. Compare sterility phenotypes of progeny with those of original pistillate parents of BC1's. Evaluate plant vigor, tuber weight and number, tuber quality, disease sensitivities, and fertilities of inbred and outbred derivatives of BC1's. Isolate and digest mtDNA, and use probes to compare electrophoretic banding patterns of the commercial potato with those of its putative ancestors for analysis of its evolution, with those of BC1's for stability determination, and with putative cybrids to determine possible relationship between mtDNA and expressions of cytoplasmic sterilities.

Analysis of growth Progress: 88/01 to 88/12. and tuber production of two generations of plants were carried out to discriminate among the possibilities that reciprocal differences resulted from maternal effects, paternal effects, or both. Founder parents were classified as strong maternal or paternal parents. At the seedling stage strong paternal \times strong paternal and strong maternal \times strong maternal parents exceeded the other possible combinations, suggesting that both paternal and maternal effects were occurring. Vigor of top growth of plants in the field and tuber production consistently showed strong paternal x strong paternal progenies significantly exceeded other combinations. The results suggest that when reciprocal differences occur they are caused by paternal effects, most likely competition among pollen tubes. Paternal effect can have a substantial influence on vigor and yield, and so should play a role in potato breeding programs. A method has been developed that is usually successful for the isolation and restriction fragment analysis of mitochondrial DNA of potato plants. We have analyzed the fragments of a sampling of plants of S. tuberosum ssp. tuberosum and ssp. andigena using the restriction endonucleases Hind III and Sst-1. There are band polymorphisms within both subspecies and a number of bands consistently show differences between the two subspecies.

Publications: 88/01 to 88/12
AMOAH, V., GRUN, P. 1988. Cytoplasmic
 substitution in 'Solanum' I. Seed
 production, germination, and sterilities of
 reciprocal backcross generations. Potato
 Res. 31:113-119.

AMOAH, V., GRUN, P., HILL, R.R., JR. 1988.
Cytoplasmic substitution in 'Solanum' II.
tuber characteristics of reciprocal
backcross progeny. Potato Res. 31:121-127.

11.013 CRISO134836 STRUCTURE AND FUNCTION OF SELF-INCOMPATIBILITY GENES: A BIOTECHNOLOGICAL APPROACH

FLORES H; KAO T H; Plant Pathology; Pennsylvania State University, University Park, **PENNSYLVANIA** 16802.

Proj. No.: PENO2997 Project Type: HATCH Agency ID: CSRS Period: O1 JUL 88 to 30 JUN 91

Objectives: Isolate and sequence the S-genes coding for the S-proteins of the gametophytic self-incompatibility system in three genera of the Solanaceae family; identify the regulatory elements of these S-genes which control their tissue-specific expression; transfer a functional S-gene into a self-compatible mutant to test whether the mutant's behaviors can be reversed back to the wild-type self-incompatible; develop an in vitro transformation and flowering system to study the expression and function of the S-gene.

Approach: Appropriate laboratory procedures will be used to accomplish the stated objectives.

Progress: 88/07 to 88/12. We have established optimal conditions for the 'in vitro' culture of tissue from 'Nicotiana alata' and 'Petunia inflata'. Using the standard Murashige and Skoog medium, supplemented with benzyl-adenine (1 mg/ml), we regenerated plantlets from leaf disc explants. This system is now being used to raise transgenic plants, with a view to studying the molecular biology of the self-incompatibility genes (S alleles). A cDNA clone of the Sz allele of 'N.alata' was ligated into the vector pBI121, 3' to a CaMV \$35 promoter and \$' to GUS, a reporter gene. This construct was introduced into 'Agrobacterium tumefaciens' (LBA4404) by triparental mating, and the bacteria then used to infect 'N. tabacum' leaf discs. Shoots were regenerated on selection plates (the plasmid confers kanamycin resistance) and are being tested for integration of Sz into the host genome. 'Agrobacterium' containing pBI121, and pBI121::Sz, was also used to infect leaf discs of 'N.alata' bearing another S allele (S(f11)). Another vector, pPCV702, which lacks the GUS gene but contains the CAMV \$35 promoter and confers kanamycin resistance, is being used to raise transgenic plants. The cDNA clone of the 'Petunia' S(2) allele was cloned into this vector, and transformants containing 'sense' and 'anti-sense' configurations of the clone are being identified. 'Agrobacterium' containing these constructs will be used to infect 'Petunia' leaf discs.

Publications: 88/07 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

11.014 CRISO034131 REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO

PARK W D: Biochemistry & Biophysics: Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6769 Project Type: CRGO Agency ID: CRGO Period: 15 SEP 83 to 30 JAN 86

Objectives: Project 8300489. In this project we will look in more detail at the regulation of the major tuber protein genes. cDNA clones will be used to examine the metabolism of the tuber protein mRNAs during normal tuber development and also in other tissues such as stems and leaves under both inducing and noninducing conditions.

Approach: The genes for patatin will be isolated by cloning in lambda phage vectors and their structures will be determined. The relationship of gene structure and function will then be examined by looking at DNAse I sensitivity and methylation patterns.

Progress: 83/09 to 86/12. We have isolated and characterized a number of genomic clones of the major potato tuber protein, patatin. These clones consist of two types, one of which (Class II), contains a 22 bp insertion in the 5' untranslated region. The genomic clone pPS20, which does not contain the insert (Class I), has been completely sequenced. It contains 6 introns and codes for an mRNA that is identical to our previously characterized cDNA clone pGM203. The 5' flanking region of three other genes without inserts has also been examined and found to be highly homologous. Genes without the insertion are expressed in tubers and can be induced to be expressed in stems and petioles, but are not normally expressed in roots. The first two Class II genes that we examined appear to be pseudogenes since they contain stop condons and we are unable to get complete protection in \$1 experiments. However, based on primer extension experiments, we have found that insert containing mRNAs are expressed tubers and, at a lower level, in roots. Interestingly, we have also found patatin related sequences in tomato. These appear to be Class II genes since they are expressed in roots and contain the 22 bp insertion.

Publications: 83/09 to 86/12 HANNAPEL, D.J., MILLER, J.C., Jr., and PARK, W.D. Regulation of Potato Tuber Protein Accumulation by Gibberellic Acid. Plant Physiol. 78, 700-703. (1985). HANNAPAL, D.J. 1985. Hormonal Regulaton of Tuber Protein Synthesis. Ph.D. Thesis, Purdue University. PIKAARD, C.S. 1985. Molecular Characterization of the Patatin Gene Family of Potato. Ph.D. Thesis, Purdue University. PIKAARD, C.S., MIGNERY, G.A., DIN POW MA, STARK, V.J. and PARK, W.D. 1986. Sequence of Two Apparent Pseudogenes of the Major Potato Tuber Protein, Patatin. Nucleic Acids Research 4:5564-5566. BOURQUE, J.E., MILLER, J.C. and PARK, W.D. 1987. Use of an In Vitro Tuberization System to Study Tuber Protein Gene Expression. In Vitro (in press).

PIKAARD, C.S., BURSCA, J.S., HANNAPEL, D.J., MIGNERY, G.A. and PARK, W.D. The Major Potato Tuber Protein, Patatin, is Expressed in Roots: Root Transcripts Contain a 22 Nucleotide Insertion.

11.015 CRISO034270 DIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES

RYAN C A: Inst of Biological Chemistry: Washington State University, Pullman, WASHINGTON 99164.

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Proj. No.: WNPO0697 Project Type: CRGO Agency ID: CRGO Period: 15 AUG 84 to 31 AUG 85

Objectives: PROJ 8400661. This research proposal is designed to establish whether the same complement of isoinhibitor species of Inhibitor I, II and CPI that are present in potato tubers are the same as those that are wound-induced in potato leaves, or if different complements of isoinhibitors are present in each tissue. These results will be the basis for a second phase of research that will be designed to identify whether the potato genes encoding Inhibitors I, II and CPI, that we have already isolated, are developmentally or environmentally regulated, or both, and to provide inhibitor genes with altered regulatory characteristics with which to study their structure-function of relationships.

Approach: Using cDNA probes already constructed for Inhibitors I and II, wound-induced inhibitor mRNA will be isolated by hybrid selection. These mRNAs will be used to construct libraries of wound-induced Inhibitor I and II cDNAs. Oligonucleotide probes will be constructed from known sequences in non-coding variable regions of genomic Inhibitor I and II clones to identify whether genes we have isolated are indeed wound-induced genes. Similar experiments using mRNA from potato tubers will be carried out in order to distinguish wound-induced genes from developmental genes.

Progress: 86/01 to 86/12. The primary structure of the wound-inducible trypsin inhibitor from alfalfa (ATI) establishes it as a member of the Bowman-Birk proteinase inhibitor family. The time course of induction of ATI in alfalfa following wounding is similar to the induction of the nonhomologous proteinase Inhibitors I and II in tomato and potato leaves, and, like Inhibitors I and II, ATI is induced to accumulate in excised leaves supplied with the proteinase inhibitor inducing factor from tomato leaves. ATI is the first Bowman-Birk inhibitor that has been found in leaves and is the only member of this family known to be regulated by wounding. Would-regulated systems are therefore present in Solanaceae and Leguminosae plant families that possess a common fundamental recognition systems in response to pest attacks. These same three inhibitors are found developmentally regulated in storage organs in potato and legume plants. The construction of alfalfa genomic library was completed in order to isolate the ATI gene. A partial Bowman-Birk

cDNA clone was obtained from Dr. Brian Larkins of Purdue University and is in use as a hybridization probe to identify the gene. Proteinase Inhibitor I protein was found in fruit of the common tomato Lycopersicon esculentum, but in relatively low concentrations (less than 1% of the soluble proteins). However, a cross of L. esculentum with a primitive species, L. purivianum, Inhibitor I protein comprised over 30% of the soluble proteins of the fruit. Another species,

Publications: 86/01 to 86/12 BROWN, W.E., TAKIO, K., TITANI, K. and RYAN, C.A. 1985. Wound-induced trypsin inhibitor in alfalfa leaves: Identity as a member of the Bowman-Birk inhibitor family, Biochemistry 24:2105-2108. RYAN. C.A., BISHOP, P.D., GRAHAM, J.S., MAYER-BROADWAY, R. and DUFFEY, S.S. Plant and fungal cell wall fragments activate proteinase inhibitor genes for plant defense. J. Chem. Ecol., in press. LEE, J.S., BROWN, W., PEARCE, G., DREHER, T.W., AHERN, K.G., PEARSON, G.D. and RYAN, C.A. Complete sequence of a wound-inducible preteinase Inhibitor I gene in tomato. Proc. Natl. Acad. Sci. USA, in press. BROWN, W., GRAHAM, J.S., LEE, J.S., RYAN, C.A. Regulation of synthesis of proteinase inhibitors in food plants. In nutritional and toxological significance of enzyme inhibitors in foods (M. Freidman ed.), Plenum Press, NY, in press.

11.016 CRISO090986 STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS

RYAN C A; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO1791 Project Type: HATCH Agency ID: CSRS Period: O1 SEP 85 to 31 AUG 90

Objectives: The objectives of this research are to understand the biochemical and molecular biological basis of insect-induced synthesis and accumulation of proteinase inhibitor proteins in plants. The complete chemical nature of the wound signals will be sought and their mechanisms of release, transport, intracellular recognition and gene activation will be studied. The structure and organization of the inhibitor genes will be investigated and the wound-induced promoter regions characterized. These promoters will be employed to improve the natural defenses of important crop plants.

Approach: Modern biochemical, immunological and molecular biological techniques will be employed, including recombinant DNA technology.

Progress: 88/01 to 88/12. Two 5' regions of the wound-inducible potato inhibitor II gene that regulate wound-induction have been identified by deletion analysis in collaboration with Dr. Gyn An. These regions, near 650 bp and 150 bp upstream from the translation initiation codon, were shown by gel

retardation assays in this laboratory to be the only regions that bind to specific tomato leaf wound-inducible nuclear proteins. One of these trans-factors has been partially purified and has a Mr of 27 kDa. The wound-inducible expression of a fused Inhibitor II-CAT gene in tobacco was shown to be enhanced over 50-fold by sucrose or other metabolizable sugars. This increase is due to transcriptional control, indicating that mRNA synthesis is somehow being regulated by a sucrose-derived molecule. A cDNA coding for wound-inducible trypsin inhibitor in alfalfa leaves (called ATI, a member of the Bowman-Birk inhibitor family) was isolated and characterized. The gene has also been isolated and is currently being characterized. A strongly expressed Inhibitor I gene has been isolated from the DNA of a wild species of tomato, L. peruvianum. This Inhibitor I gene is being introduced into the modern tomato where it is not expressed in fruit. The expression in modern fruit could allow the development of a fruit expression system. Oligosaccharides that induce the accumulation of proteinase inhibitors in plants have been shown to cause the enhanced phosphorylation of plasma membrane proteins from potato and tomato.

Publications: 88/01 to 88/12

CLORE, G.M., GRONEBORN, A.M., NILGES, M. and RYAN, C.A. (1988). The Three-Dimensional Structure of Potato Carboxypeptidase Inhibitor in Solution: A Study Using Nuclear Magnetic Resonance, Distance Geometry and Restrained Molecular Dynamics. RYAN, C.A. and AN, G. (1988). Molecular

Biology of Proteinase Inhibitors in Plants.
Plant, Cell and Environment 11:345-349.

- RYAN, C.A. (1988). Oligosaccharide Signalling for Proteinase Inhibitors in Plant Leaves. In "Advances in Phytochemistry" (Conn, E., ed.) Vol. 22, Plenum Press, NY, pp. 163-180.
- PEARCE, G., LILJEGREN, O. and RYAN, C.A. (1988). Proteinase Inhibitors in Fruit of the Wild Tomato Species L. peruvianum: A possible Mechanism for Plant Protection and Seed Dispersal. Planta 175:527-531.
- AN, G., THORNBURG, R.W., JOHNSON, R., HALL, G. and RYAN, C.A. (1988). A Possible Role for 3' Sequences of the Wound-Inducible Potato Proteinase Inhibitor IIK Gene in Regulating Gene Expression. In "NATO Conference Proceedings".
- RYAN, C.A. (1988). Proteinase Inhibitor Genes: Strategies for Manipulation to Improve Natural Plant Defense. BioEssays (in press).
- GREENBLATT, H.M., RYAN, C.A. and JAMES, M.N.G. (1988). Structure of the Complex of Streptomyces Griseus Proteinase B andPolypeptide Chymotrypsin inhibitor I from Russet Burbank Potato Tubers at 2.1: A Resolution (in Press).

11.017 CRISO143081 SYSTEMATICS OF POTATOES AND THEIR WILD RELATIVES (SOLANUM SECT. PETOTA)

SPOONER D M; Agricultural Research Service, Madison, WISCONSIN = 53702 .

Proj. No.: 3655-21000-004-00D

Agency ID: ARS

Petota.

Project Type: INHOUSE Period: 01 OCT 88 to 31 MAR 89

Objectives: To develop species boundaries and systematic relationships among and between wild and cultivated potato species (Solanum sect. Petota), and to determine the relationships of this group to near relatives outside of thissection. Also to test the extent and degree of natural hybridization in Solanum sect.

Approach: Restriction mapping techniques using both organellar and nuclear DNA, as well as isozymes, will be used to construct phylogenetic trees and similarity matrices using modern, computer-assisted numerical taxonomy techniques. Crossing studies also will be used to resynthesize putative hybrid taxa.

Progress: 88/10 to 88/12. Two hundred fifty accessions of 120 Solanum sect. Petota taxa. were collected. DNA has been extracted and purified from 150 of these. An initial study using cpDNA nrDNA and 175-285 nrDNA of 16 accessions of Solanum sect. Petota has been completed and is being prepared for publication, and a follow-up study on other South American relatives, using 87 accessions, is in progress. The diploid hybrid speciation hypothesis of Solanum raphanifolium was reexamined using S. canasense, S. megistacrolobum, and its presumed hybrid derivative S. raphanifolium; other related species in ser. Megistacrolobum and ser. Tuberosum; and related species in ser. Megistacrolobum and ser. Tuberosum; and related outgroup species in ser. Etuberosum. The results show considerable restriction site variation within this group, but of a degree that is interpretable with the sequential probing methodology. The cpDNA and rnDNA data are concordant and indicate that S. raphanifolium and S. canasense are related, but without the contribution of genes from S. megistacrolobum as was hypothesized by Ugent on the basis of morphological intermediary and inference from distributional data. Another study is in progress on the cpDNA divergence of 30 Mexican and South American species, and another study is in progress on the relationships of Solanum sect. Petota to outgroup relatives in Solanum and Lycopersicon.

Publications: 88/10 to 88/12
SPOONER, D.M., SYTSMA, K.J., SMITH, J.F.,
STAUB, J.E., and KNERR, L. 1988. A
re-examination of diploid hybrid speciation
in Solanum sect. Petota. Amer. J. Botany
75:208. Abstract.

11.018 CRISO049729
DEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR
INCOR- PORATING DISEASE RESISTANCE INTO
POTATOES

HANNEMAN R E; HELGESON J P; DEZOETEN G A; Plant Pathology; University of Wisconsin, Madison, **WISCONSIN** 53706.

Proj. No.: 3655-21000-002-01S

Project Type: COOPERATIVE AGREE. Agency ID: ARS Period: 27 SEP 84 to 30 SEP 88

Objectives: To develop molecular biology means to incorporate useful disease resistance into potato.

Approach: To make gene libraries for resistant characteristics; to apply and develop molecular biology methods for messenger RNA isolation, cloning techniques synthesize and study proteins generated by messages, etc.; to transfer vital genes to the host to induce resistance; to transfer resistant genes from resistant plants into cells of susceptible plants, and to evaluate progency from experiments for resistance to common viruses.

Progress: 87/01 to 87/12. Using potato virus Y (PVY) as model virus for devising a cross protection-based system to induce resistance. Have developed method for routine isolation of PVY yielding 30-50 mg. per kg. of plant tissue. The protein coat has been isolated and purified using SDS-PAGE and was confirmed by Western blotting and immunology. The first 20 amino terminal residues were sequenced. The sequence will be used to locate the coat protein gene once the nucleic acid sequence for the genome is determined. Large quantities of PVY mRNA has been isolated using recently reported procedures. The integrity of the RNA has been verified in a rabbit reticulocyte cell-free system. One major product appears to be the PVY coat protein. The PVY RNA has been used to synthesize single stranded cDNA, and the system for its synthesis has been optimized. Physical maps of the cDNA have been generated using restriction endonucleases. Mapping and sequencing information is being used to determine the concentration of the cDNA in the vector

Publications: 87/01 to 87/12

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NO PUBLICATIONS REPORTED THIS PERIOD.

11.019 CRISO027442 FUNDAMENTAL RESEARCH TO DEVELOP CONTROL MEASURES FOR BACTERIAL PATHOGENS OF THE POTATO

SEQUEIRA L; KELMAN A; Plant Pathology; University of Wisconsin, Madison, **WISCONSIN** 53706.

Proj. No.: WISO6070 Project Type: STATE Agency ID: SAES Period: 01 JAN 86 to 30 JUN 92

Objectives: To determine, under highly controlled conditions, the environmental parameters that affect biological control of bacterial wilt of potatoes. To determine the biochemical nature of the host and pathogen signals that result in induction of the hypersensitive response in tissue cultures of

Solanum phureja inoculated with Pseudomonas solanacearum. To obtain monoclonal antibodies against Pseudomonas solanacearum race 3, with the ultimate aim of developing a rapid diagnostic kit.

Approach: Parameters to be studied in relation to biological control with bacterial strain B82 are: soil moisture, light, temperature, numbers of root knot nematodes in the soil, aggressiveness of the strain used in the challenge inoculations, and level of intrinsic tolerance of the test potato. Diffusible factors, produced by P. solanacearum when in contact with plant tissue cultures of resistant cultivars and associated with induction of a hypersensitive reaction, will be purified and their properties determined. Monoclonal antibodies to race 3 of P. solanacearum will be obtained from hybridomas prepared from the spleen cells of mice tolerized to race 1 and immunized with race 3 cells.

Progress: 88/01 to 88/12. We have continued to explore the possible development of diagnostic probes for race 3 of P. solanacearum based on the restriction fragment length polymorphisms (RFLP) of the genomic DNA of this species. RFLP analysis involving nine DNA probes, seven of which encode information required for virulence and the hypersensitive response (HR), was used to study the relationships among 62 strains, representing three races and five biovars. When Southern blots of EcoR1- or EcoR1 and BamH1-digested genomic DNA were probed with the nine unique EcoR1 cloned fragments from strains K60 or K2R, 28 distinct RFLP patterns were identified. Similarity coefficients revealed two major divisions: division I contains all members of race 1 biovars 3, 4, and 5; division II contains all members of race 1 biovar 1 and races 2 and 3. Similarity coefficients within divisions I and II were 78% and 62%, respectively. In contrast, the similarity coefficient between the two divisions was only 13.5%. Division II is composed of five distinct subdivisions corresponding to race 1 biovar 1, race 3, and three subdivisions of race 2. These subdivisions correspond to strains with distinct host ranges and geographical origins. We identified three dissimilar RFLP groups within race 2, each of which was associated with a different epidemic in a different geographic location.

Publications: 88/01 to 88/12

MCLAUGHLIN, R.J. and SEQUEIRA, L. 1988. Evaluation of an avirulent strain of Pseudomonas solanacearum for biological control of bacterial wilt of potato. Am. Potato J. 65:255-268.

MCLAUGHLIN, R.J. and SEQUEIRA, L. 1989. Phenotypic diversity of Pseudomonas solanacearum from a single potato field. Plant Disease (in press).

MCLAUGHLIN, R.J., SEQUEIRA, L. and WEINGARTNER, D.P. 1988. Biocontrol of bacterial wilt with an avirulent strain of Pseudomonas solanacearum: interactions with root knot nematodes. Am. Potato J. 65: (in press).

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COOK, D., BARLOW, E. and SEQUEIRA, L. 1989. Genetic diversity of Pseudomonas solancearum: detection of restriction fragment length polymorphisms with DNA probes that specify virulence and the hypersensitive response.

CM 12 VEGETABLES

12.001 CRISO137812 THE MOLECULAR BASIS OF BLACK ROT OF CRUCIFERS

SHAW J J; Botany & Microbiology; Auburn University, Auburn, ALABAMA 36830.

Proj. No.: ALAOO787 Project Type: HATCH Agency ID: CSRS Period: 10 APR 89 to 30 SEP 93

Objectives: Mutagenesis of Xanthomonas, identification of genes involved in plant colonization. Study of gene expression and regulation.

Approach: Transposon mutagenesis to make mutants, cosmid gene library and gene complentation to identify genes, restriction mapping and sequencing of DNA to characterize genes, reporter genes to study gene expression (in planta).

12.002 0090545 EXPRESSION OF MELANIN IN PLANTS - MONITORING GENE EXPRESSION

BOHNERT H J; Biochemistry; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-136334-H-49-081

Project Type: HATCH Agency ID: CSRS Period: 01 OCT 89 to 30 SEP 92

Objectives: To construct genes that express a protein that leads to a color change in specific plant organs. Plant transformation and monitoring of gene expression.

Approach: Modified genes will be constructed using different promoter regions that drive expression of a polyphenoloxidase coding region. These genes will be stably transformed into ornamental species. Biochemical characterization of the expressed protein and analysis of the products of enzyme activity will be performed.

Progress: 88/01 to 88/12. We are studying the genome and gene organization of the "ancient" plastid found in Cyanophora paradoxa as a model for shuttling genes and gene products between cellular compartments.

Publications: 88/01 to 88/12

BREITENEDER, H., SEISER, C., LOEFFELHARDT, W., MICHALOWSKI, C.B., BOHNERT, H.J. (1988). Physical map and gene map of cyanelle DNA from the second known isolate of Cyanophora paradoxa (Kies strain). Curr. Genetics 13:199-206.

MICHALOWSKI, C.B., RICKERS, J., RAMAGE, R.T., SCHMITT, J.M., BOHNERT, H.J. (1988). Functional replacement in bacteria of a higher plant gene for phosphoenol pyruvate carboxylase. In: Physiol. Suppl. 86:16 (Abstract).

JANSSEN, I., JAKOWITSCH, J., MICHALOWSKI, C., BOHNERT, H.J., LOEFFELHARDT, W. (1988). Sequence Analysis of the Cyanelle PSBA-Gene from Cyanophora paradoxa. The Second International Congress of Plant Molecular Biology, Jerusalem, Nov. 13-18.

12.003 CRISO098702 DEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS

VIERLING E; Biochemistry; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-136340-H-49-012

Project Type: HATCH

Agency ID: CSRS Period: 01 JUL 86 to 30 SEP 89

Objectives: To determine if plants synthesize additional high molecular weight chloroplast heat shock proteins. If these proteins are found, work will be initiated to obtain corresponding cDNA clones. To determine if chloroplast heat shock proteins are expressed during embryogenesis or germination in the absence of stress. To determine if heat shock proteins can be induced by heat even during embryogenesis and germination. All work is being performed with Pisum sativum cv. "Little Marvel.

Approach: The presence of high molecular weight heat shock proteins will be analyzed by in vitro transport of proteins into isolated chloroplasts. Expression of heat shock proteins will be analyzed by Northern blotting using cloned cDNA probes. When antibodies are available, these will be used to detect proteins.

Progress: 88/01 to 88/12. Results of this research indicate that heat shock proteins function during seed development in the absence of heat stress. Using Pisum sativum as a model system, we have isolated cDNAs encoding HSP70 and four gene families of low molecular weight HSPs. Embryos from seeds developing in the absence of heat stress exhibit significant levels of mRNA for each of these HSPs. We are continuing to characterize the temporal and spatial regulation of HSP mRNA expression during seed development. DNA sequence analysis of one of the low molecular weight HSPs (HSP18) is complete and sequencing of the other clones is in progress. Using HSP70 antibodies, HSP70 proteins can be detected in normal seeds. Several different HSP70 proteins have been detected, one of which is seed specific. Additional cDNA clones for each of the HSP70s have been isolated and are being characterized. To determine if the other HSPs are also synthesized during development, antibodies against each low molecular weight HSP are being produced. HSP18 has been expressed as a fusion protein in E. coli, and is being used for antibody production. To test for similar responses in other plants, cDNAs for heat shock proteins have been isolated from Arabidopsis thaliana. Studies of HSP expression during plant development should yield new insights into HSP function and basic developmental processes.

Publications: 88/01 to 88/12

VIERLING, E. and DEROCHER, A. (1988). Heat shock mRNAs are expressed during seed development. Plant Physiol. 865:25 (Abstract).

VIERLING, E., NAGAO, R.T., DEROCHER, A.E., HARRIS, L.M. (1988). A Chloroplast-localized Heat Shock Protein is a Member of a Eukaryotic Superfamily of Heat Shock Proteins. EMBD J. 7: 575-581.

12.004 CRISO142131
MOLECULAR BIOLOGY OF FLOWER DEVELOPMENT IN
AGRONOMICALLY IMPORTANT PLANTS

MCCDRMICK S; YAMAGUCHI J; Agricultural Research Service, Albany, **CALIFORNIA** 94710. Proj. No.: 5335-22230-002-00D

Project Type: INHOUSE

Agency ID: ARS Period: 15 JUL 87 to 14 JUL 92

Objectives: Isolate flower-specific genes & gene promoters (control regions) in order to modify gene expression during flowering.

Approach: Characterize gene expression of anther-specific genes during flower development and pollen germination. Isolate promoter region(s) of selected anther-specific genes, construct chimeric genes (with coding regions expected to affect flowering) and transfer to plants (via Agrobacterium) inorder to modify flower development. Develop technologies to clone nuclear male-sterile genes.

Progress: 88/01 to 88/12. We previously reported the isolation and preliminary characterization of cDNAs that are specifically expressed in tomato anthers. Some of these clones are expressed as early as tetrad stage of microsporogenesis, but all are maximally $% \left(\frac{1}{2}\right) =\frac{1}{2}\left(\frac{1}{2}\right) ^{2}$ expressed in pollen. In situ hybridizations with these clones show that the genes are expressed in both the pollen and the anther wall. We have sequenced these clones. Two of the clones show no homology to sequences in the databases, but two show significant homology to the pectate lyase genes of Erwinia. Using RFLP analysis we showed that these two genes are linked. We predict that they are secreted proteins. We have isolated and sequenced the promoter regions of three of these pollen-expressed genes. More detailed studies of these promoters using deletion analysis and gel retardations are in progress. We have constructed promoter-fusions with reporter genes, and have introduced these constructs into tobacco and tomato. We determined that a 1.4kb region of one of the promoters, and a 0.5kb region of another, are sufficient to direct reporter gene expression in a pollen-specific manner. We have also tranformed plants with promoter fusions to the auxin and cytokinin genes. Additionally, antisense constructs of the pollen-specific genes have been made and introduced into plants, in order to test an altered flower or pollen phenotype.

Publications: 88/01 to 88/12

FISCHHDFF, D.A., BOWDISH, K.S., PERLAK, F.J., MDRRDNE, P.G., MCCDRMICK, S.M. 1988. Insect tolerant transgenic tomato plants. Bio/Technology 5: 807-814.

NELSON, R.S., MCCORMICK, S.M. 1988. Virus tolerance, plant growth, and field performance of transgenic tomato plants expressing coat protein from tobacco mosaic virus. Bio/Technology.

CATLIN, D., DCHOA, O., MCCDRMICK, S. and QUIRDS, C.F. 1988. Celery transforma- tion by Agrobacterium tumefaciens: Cytological

and genetic analysis of trangenic plants. Plant Cell Reports.

12.005* CRISO142123
MOLECULAR BIOLOGY OF PLANT SENESCENCE

THEDLDGIS A; TBD; TBD; Agricultural Research Service, Albany, **CALIFORNIA** 94710.

Proj. No.: 5335-23410-002-00D

Project Type: INHDUSE Agency ID: ARS Period: 15 JUL 87 to 31 JAN 88

Objectives: Isolate & structurally characterize genomic sequences to early auxin- inducible mRNAs from pea tissue. Regulate expression of the hormonally regulated genes in vivo & in isolated pea nuclei. Purify & localize at the subcellular level the proteins coded by the auxin-regulated genes.

Approach: Genomic libraries will be constructed into the cloning vector EMBL 3 using DNA from etiolated pea seedlings. The genomic sequences of the early auxin regulated mRNAs will be isolated by plaque filter hybridization using the already isolated cDNA clones pIAA4/5 & pIAA6 as probes. The organization & structural analysis of the genes will be investigated in detail. To deter- mine whether auxin acts at the transcriptional or post-transcriptional level, the stability of the inducible mRNAs will be examined in vivo. Theseexperiments will be supplemented with in vitro transcription in isolated nuclei. Finally, the auxin cDNA clones will be introduced into gamma expression vectors to produce fused proteins with beta-galactosidase. Anti-bodies directed toward the hybrid proteins will then be used to purify the proteins, localize them at the subcellular level & determine the kinetics of their accumulation during cell growth.

Progress: 88/01 to 88/12. During the last year we were able to isolate a DNA sequence complementary to the mRNA of Acc Synthase in Cucurbita Fruit. The experimental approach used was unique and allowed us to clone the Acc synthase rapidly. The experimental evidence indicates that the Acc Synthase gene is transcriptionally regulated by auxin and Li ions. More recently we were able to isolate genomic sequences to Cucurbita as well as to tomato Acc synthase. Structural characaterization of the genes is in progress.

Publications: 88/01 to 88/12

THEDLDGIS, A. 1988. Regulation of Gene Expression by Idoleacetic Acid in Pea Epicotyl Tissue. In the Biomech. Reg. Growth & Dev. Keys to Prog. Beltsville Sym XII. Editors, Steffens, GL and Rumsey TS. Kluwer Academic Publisher.

THEDLDGIS, A. 1988. Auxin Reg. Gene Expression in Pea. In "Plant Biotechnology" Editors, Kung,SD and Arntzen, CJ. Publishers: Butterworths. To appear in the Buttersworths Biotech. Series. J.Davies, Biogen Series Editor. In Press.

THEOLOGIS, A. 1988. Molecular Cloning of Early IAA Reg. mRNA in Pea. Proceedings of the Intnl. Symp. on "The Physiology and Bioch. of Auxins in Plants", Liblice, Czechoslovakia, 09/28-10/2/87. Editors: Kutacek, M., Bandurski, R. and Kerkula REDDY, S., KOSHIBA, T., THEOLOGIS, A. and POOVIAH, B.W. 1988. The Effect of Calcium Antagonists on Auxin-Induced Elongation and on the Expression of Two Auxin-Regulated Genes in Pea Epicotyls. Plant Cell Physiology 29: 1165-1170.

SATO, T. and THEOLOGIS, A. 1988. Cloning the mRNA of ACC Synthase: The key regulatory enzyme in the Ethylene Biosynthetic Pathaway in Plants: Scienc.

SATO, T. and THEOLOGIS, A. 1988. ACC Synthase from Cucurbita: Purification Properties and Antibody Production. Submitted to J.B.C.

12.006 CRISO034268 CHILLING AND GENE EXPRESSION IN FRUIT

FISCHER R L; Molecular Plant Biology; University of California, Berkeley, **CALIFORNIA** 94720.

Proj. No.: CA-B*-MPB-4672-CG Project Type: CRGO Agency ID: CRGO Period: 15 JUL 84 to 31 AUG 87

Objectives: PROJ 8400383. Many fruits and vegetables of tropical or subtropical origin suffer severe damage when exposed to low, nonfreezing temperatures. Although a great deal is known about the metabolic and physiological consequences of chilling, very little is known about the role gene expression plays in the process. To address this issue, fruit from both chill-sensitive and chill-solerant lines of tomato (Lycopersican exculentum) will be analyzed for changes in gene expression that are induced by chilling.

Approach: Sensitive cascade hybridization techniques will be employed to isolate cDNA clones of mRNAs that accumulate when either sensitive or tolerant fruit is exposed to low temperatures. Once obtained, the cDNA clones will be sued to measure quantitatively the alterations in gene expression that occur in chilled fruit and in 1ther plant organs. The results obtained from these experiments will further our understanding of how chill-sensitive and chill-tolerant plants respond to low, nonfreezing temperatures.

Progress: 84/07 to 87/08. Many plants of tropical and subtropical origin sustain damage when exposed to low, non-freezing temperature. We have found that the response to this chilling stress involves changes in gene expression. We have studied the induction of gene expression in response to low temperature stress by cloning mRNAs that accumulate when unripe tomato (Lycopersicon esculentum) fruit are incubated at 4 C. Two cloned mRNAs accumulate relatively rapidly in response to cold treatment. Significant levels of these mRNAs were not detected during fruit ripening at normal temperature and sustained gene expression requires continuous cold treatment. Furthermore, the level of gene expression in cold tolerant (hybrid L. esculentum/L. pimpinellifolium) fruit is different from that in cold sensitive (L. esculentum) fruit. DNA sequence analysis indicates that one mRNA encodes a polypeptide wita region that is

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homologous to the plant thiol proteases actinidin and papain, and to the animal thiol protease cathepsin H. We conclude from these experiments that low temperature selectively induces the expression of specific genes, and that one such gene encodes a thiol protease.

Publications: 84/07 to 87/08 SCHAFFER, M. and FISCHER, R.L. 1988. Analysis of mRNAs that accumulate in response to low temperature identifies a thiol protease gene in tomato. Plant Physiology. In press.

12.007 CRISO134506 ISOLATION OF A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO

WILLIAMSON V M; Nematology; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-NEM-4920-H Project Type: HATCH Agency ID: CSRS Period: 12 APR 88 to 30 SEP 92

Objectives: To clone the Mi gene which confers resistance to rootknot nematodes in tomato. To investigate the mechanisms of resistance. To transfer Mi to other plant species.

Approach: We will clone Mi by virtue of its linkage to the gene Aps-1 which encodes acid phosphatase-1. Acid phosphatase-1 will be purified and used to produce antibody and obtain peptide sequence. We will screen a cDNA library for a clone of Aps-1 and use this clone to "walk" to Mi. We will transform susceptible tomato with Mi candidates and assay for resistance to nematodes. An approach to Mi using transposon tagging is also planned. The level, localization and regulation of Mi expression will be determined. Mi will be transferred to other plant species using Agrobacterium based vectors.

Progress: 88/04 to 88/12. Progress has been made toward our goal to clone the tomato gene Aps-1 which encodes acid phosphatase-1. We have purified the enzyme acid phosphatase-1 from cell suspension culture of the nematode resistant tomato cultivar VFNT cherry. We have cleaved the purified protein with proteases and have obtained amino acid sequence information on some of the peptides obtained. This sequence information was used to generate three oligonucleotide probes and these were used to screen a VFNT genomic library. Seven candidate clones have been obtained and are being analyzed to see if they correspond to Aps-1. The Aps-1 clone will be used as a starting point in our attempt to clone the nematode resistant gene Mi of tomato by chromosome walking.

Publications: 88/04 to 88/12
No publications reported this period.

12.008 VARIATION IN LETTUCE DOWNY MILDEW

CRISO096012

MICLELMORE R W; Regents of The University; University of California, Davis, CALIFORNIA 95616.

Proj. No.: CA-D*-VCR-4700-CG Project Type: CRG0 Agency ID: CRG0 Period: O1 JUL 85 to O1 JUL 88

Objectives: Proj 8500199. We will develop detailed genetic maps of Lactuca sativa and a pathogen, Bremia lactucae using restriction fragment length polymorphisms. Plasmid clones containing low copy number genomic sequences and cDNA clones will be used as probes. Approximately 60 probes will then be selected for each species as molecular markers spaced ca. 20 cM throughout each genome.

Approach: These will be used to investigate the evolution of genes for disease resistance and virulence and to characterize the numbers, distributions and interactions of genes determining resistance and virulence in host and pathogen. The studies further develop lettuce downy mildew as a model system for the study of plant disease and are prerequisites to cloning resistance genes.

12.009 CRISO133192 POLYGALACTURONASE: ENZYME FUNCTION AND GENE REGULATION DURING FRUIT RIPENING

BENNETT A B; FISCHER R L; Vegetable Crops; University of California, Davis, CALIFORNIA 95616.

Proj. No.: CA-D*-VCR-4883-CG Project Type: CRG0 Agency ID: CRG0 Period: 15 AUG 87 to 31 AUG 90

Objectives: PROJ. 8702805. Our overall goals are to determine the effect of polygalacturonase enzyme action on cell wall structure, tomato fruit softening and tomato fruit ripening, and to define the molecular processes that regulate polygalacturonase gene expression. Our specific objectives in achieving the overall goal are: To determine the effect of induced polygalacturonase gene expression on cell wall structure and fruit ripening in transgenic plants. To identify DNA sequences and cellular factors that regulate polygalacturonase gene expression.

Approach: Our approach is to construct inducible chimeric genes using recombinant DNA methods that will be introduced into and expressed in plants bearing mutations that inhibit fruit softening and ripening. Regulatory DNA sequences and cellular factors will be identified by altering nucleotide sequences and by binding of nuclear proteins.

Progress: 88/01 to 88/12. Objectives: To define the function of polygalacturonase in tomato it fruit ripening and softening, and to define the molecular events that regulate polygalacturonase gene expression. Approach: Chimeric polygalacturonase genes were constructed and transferred into ripening-impaired mutant (rin) tomato fruit.

The chimeric gene was expressed in this normally polygalacturonase null genetic background and its effect analyzed. Nuclear DNA-binding factors were assayed for binding regulatory sequences of the polygalacturonase gene. Results: Polygalacturonase expression in transgenic rin fruit resulted in pectin degradation in the cell wall but had no effect on fruit softening or ripening. DNA-binding factors were identified and their site of binding to the polygalacturonase gene localized.

Publications: 88/01 to 88/12 OELLAPENNA, D. and BENNETT, A. (1988). In vitro synthesis and processing of tomato fruit polygalacturonase. Plant Physiol. 86:1057-1063. GIOVANNONI J. J., DELLAPENNA, D., BENNETT, A. B. and FISCHER, R. L. (1989) Expression of a chimeric polygalacturonase gene in transgenic rin (ripening inhibitor) tomato fruit results in polyuronide degradation but not fruit softening. BENNETT, A. B., DELLAPENNA, D., FISHER, R. L., GIVANNONI, J. and LINCOLN, J. E. (1989). Regulation, maturation and function of tomato fruit polygalacturonase. In: Signals for cell separation (Osborne, D., ed.). Springer-Verlag. In press. BENNETT, A. B., DELLAPENNA, O., FISHER, R. L., GIVANNONI, J. and LINCOLN, J. E. (1989). Tomato fruit polygalacturonase: Gene regulation and enzyme function. In: Biotechnology and Food Quality (Shain-Dow Kung, ed.). University of Maryland. DELLAPENNA, D., LINCOLN, J. E., FISCHER, R. L. and BENNETT, A. B. Transcriptional analysis of polygalacturonase and other

12.010 CRISO098263 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY

ripening associated gene in rutger, rin,

nor and Nr tomato fruit. Submitted.

MICHELMORE R W; Vegetable Crops; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-VCR-4642-RRProject Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 90

Objectives: To isolate and to characterize genes in plants controlling nitrogen metabolism, carbon metabolism, development and resistance to pathogens.

Approach: Recombinant DNA techniques will be used to clone, characterize and reintroduce genes for disease resistance into lettuce. Transformation mediated by Agrobacterium will be used to develop a transposon mutagenesis system using maize transposable elements. This will be used to identify resistance genes. Tagged, mutated genes will be used as probes for the wild type resistance gene. Wild type resistance genes will be reintroduced in susceptible lettuce lines to test for function. Genes determining the response of plants to challenge by pathogens will be mapped using heterologous genes as probes in RFLP analysis.

Progress: 88/01 to 88/12. This section of the project involves developing techniques for cloning and characterizing genes in lettuce for resistance to the fungal pathogen, Bremia lactucae. As only limited funds have been available, much of the work has been done in conjunction with other funding. We are developing a system of transposon tagging using heterologous transposable elements. We have introduced several transposons from other species into lettuce. We have concentrated on Ac from maize and Tam3 from snapdragon due to the good results with these elements in other species. Initially, transposition was assayed by Southern analysis of the transgenic plants to detect a characteristic excision restriction fragment. We failed to detect evidence of Ac transposition in lettuce; although, when the same construct was introduced by collaborators into tomato, high frequencies of transposition were observed. Southern analysis of plants containing Tam3 were consistent with a low frequency of transposition; this is being confirmed in R(subscript 2) Progeny. More recent constructs with Ac involved a streptomycin resistance reporter gene which should only be expressed following excision of Ac from its 5' region (Courtesy J. Jones, Sainsbury Lab, UK). Of 65 R2 progeny containing this reporter construct, nearly all showed clear segregation for streptomycin resistance. This may indicate a high rate of transposition similar to that seen in other dicotyledenous species or may be due excision of Ac sequences during mRNA processing.

Publications: 88/01 to 88/12

MICHELMORE, R. W., HULBERT, S. H., LANDRY, B. S. and LEUNG, H. (1987). Towards a molecular understanding of lettuce downy mildew. In: Genetics of Plant Pathogenesis. Eds. P.R. Day and G.J. Jellis. Blackwell Scientific Pubs. Oxford.

YODER, J. F., BELZILE, K., ALPERT, K., PALYS, J. and MICHELMORE, R. W. (1987).

Mobilization of the maize transposable element, Ac, in tomato. In: Proc. Tomato Biotech. Symp., Davis. Eds. D.J. Nevins and R.A. Jones. Alan Liss, Inc., N.Y.

12.011 CRISO077803 GENETICS AND BREEDING OF COOL SEASON CROPS

QUIROS C F; Vegetable Crops; University of California, Davis, **CALIFORNIA** 95616.

Proj. No.: CA-D*-VCR-3848-H Project Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 90

Objectives: Genetic improvement of celery breeding line UC1. Screen celery germplasm for leafminer resistance. Explore the possibility of obtaining haploids in celery for the development of F(1) hybrids. Study Apium species relationships by interspecific hybridization. Development of genetic markers in celery and coles. Study the genome evolution of Brassica.

Approach: Conventional backcross program to improve our fusarium resistant line for horticultural traits. Test about 100 celery accessions in the field for leaf miner

infestation. Re-test promising lines for resistance in the laboratory. Attempt to obtain haploids in celery by anther culture. Enzyme electrophoresis and restriction fragment length polymorphism will be used for genome evolution studies in celery and coles.

Progress: 88/01 to 88/12. Further selection for Fusarium resistance was performed on our advanced celery breeding lines at several infested fields in Southern California. After three cycles of backcrossings to the variety Tall Utah 52-70R' the horticultural traits for most of these plants are approaching commercial type. Selected plants from the field trials were selfed to fix the resistance in a homozygous condition. Another selection cycle was performed this year on progenies derived from the best lines. Three lines have been identified for release in 1989. These are highly resistant to Fusarium but still deviate from ideal horticultural type. Plants from these lines should provide a good source for deriving improved varieties. Studies on the inheritance to Fusarium resistance indicates that two genes are involved, Fu1 derived from celeriac, confers a high degree of resistance, whereas Fu2 derived from the variety T.U. 52-70HK, confers a low degree of resistance. These genes seem to have codominant expression, so in order to maximize the resistance it is necessary to have double homozygous individuals for the dominant alleles. he interspecific hybridization program involving the pest and disease resistant wild species A. panul, A. chilense and A. prostratum was expanded. Tests of first and second backcross derivatives from the Septoria resistant species A. chilense did not have the high level of resistance observed in the parental wild species.

Publications: 88/01 to 88/12
QUIROS, C.F. (1987). Stalking celery diseases. Amer. Veg. Grower 35:54.
TRUMBLE, J.T. and QUIROS, C.F. (1988).
Antixenotic and antibiotic resistance in Apium species to Liriomyza trifolii (Diptera:Agromyzidae). J. Econ. Entomol. 81:602-607.

12.012 GENOME EVOLUTION IN BRASSICA

CRISO099130

QUIROS C F; Vegetable Crops; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-VCR-4719-CG Project Type: CRGO Agency ID: CRGO Period: O1 JUN 86 to 31 MAY 88

Objectives: PROJ. 8600025. The goal of this project is to study the evolution of the Brassica genome. Brassica is a complex genus including diploid and natural amphidiploid species. The diploids have genomic numbers ranging from x=7 to 11.

Approach: This study will provide fundamental information on: a) the extent of chromosomal rearrangements and duplications during changes in genome size, b) the mechanisms leading to polyploidy and aneuploidy in Brassica evolution, and c) genetic organization of the chromosomes forming the basic genomes of the

diploid species approach. For this purpose, the development of cytogenetic stocks, based on the construction of alien chromosome addition lines is proposed. This will serve to "dissect" most of the chromosomes in the different genomes, which will provide linkage information of molecular markers, such as isozymes and restriction site polymorphisms.

A series of Progress: 86/01 to 88/07. addition lines were developed from natural and artificial amphidiploids Brassica species. These are: B. campestris-oleracea from B. napus, B. campestris-oleracea from 'Hakuran' (synthetic B. napus), B. oleracea-nigra from B. carinata, Oipoltaxis erucoides-nigra from a synthetic hybrid of both species. Most of the lines carrying either B. oleracea or B. nigra additional chromosomes were characterized by species specific markers such as isozymes and RFLPs. For the RFLP characterization, B. napus and B. oleracea genomic libraries were developed. Also, ribosomal rONA polymorphism were employed successfully. Preliminary results indicate that one chromosome pair of oleracea and two pairs of B. nigra carry rRNA genes. The synteny relationships found so far for the markers of these two species demonstrate that the genomes of these two species has been reshuffled. Oifferences in synteny were also observed in the chromosomes of B. oleracea when derived from the natural amphidiploid B. napus. The results of this project demonstrate that it is possible to develop cytogenetic stocks useful for genome and genetic analysis in Brassica. In the second phase of the project, this material will be used to study more in depth the genetic organization of the Brassica genomes at the molecular level.

Publications: 86/01 to 88/07

QUIROS, C.F., OCHOA, O., KIANIAN, S.F. and OOUCHES, O.S. 1987. Analysis of the Brassica oleracea genome by the generation of B. campestris-oleracea chromosome addition lines: characterization by isozymes and rRNA genes.

QUIROS, C.F., OCHOA, O. and OOUCHES, O.S. 1988. Exploring the role of x=7 species in Brassica evolution: hybridization with B. nigra and B. oleracea. J. of Hered. 79:351-359.

QUIROS, C.F. et al. 1988. Alien addition lines in Brassica: construction, characterization and their possible applications. HortSci. 23:777 (Abs).

KIANIAN, S.F. and QUIROS, C.F. 1988. Evolutionary analysisis of the Brassica oleracea cytodeme. Genome 30:286 (Abs).

MCGRATH, M. and QUIROS, C.F. 1988.

Oevelopment and characterization of alien addition lines from synthetic Brassica napus. Genome 30:286 (Abs).

TARQUIS, A., LASSNER, M. and QUIROS, C.F. RFSIZE: A basic program to estimate ONA fragment size with a digitizer. J. Hered. (in press).

12.013 GENOME EVOLUTION IN BRASSICA

CRISO136848

QUIROS C F; Vegetable Crops; University of California, Oavis, **CALIFORNIA** 95616. Proj. No.: CA-O*-VCR-5037-CG Project Type: CRGO Agency IO: CRGO Period: O1 SEP 88 to 31 AUG 90

Objectives: PROJ. 8800301. The goal of this project is to study the evolution of the Brassica genome. Brassica is a complex genus including diploid and natural amphidiploid species. The diploids have genomic numbers ranging from x=7 to 11. This study will provide fundamental information on: a) the extent of chromosomal rearrangements and duplications during changes in genome size, b) the mechanisms leading to polyploidy and aneuploidy in Brassica evolution, and c) genetic organization of the chromosomes forming the basic genomes ofthe diploid species.

Approach: The development of cytogenetic stocks, based on the construction of alien chromosome addition lines is proposed. This will serve to "dissect" most of the chromosomes in the different genomes, which will provide linkage information of molecular markers, such as isozymes and restriction site polymorphisms. For this purpose a number of random ONA probes obtained from Brassica genomic libraries will be employed.

12.014 VEGETABLE GENETICS

CRISO087465

RICK C M; Vegetable Crops; University of California, Oavis, CALIFORNIA 95616.
Proj. No.: CA-O*-VCR-4223-H Project Type: HATCH Agency IO: CSRS Period: 20 MAY 82 to 30 SEP 88

Objectives: To investigate the basic genetics, cytogenetics, and sources of variation in a vegetable crop and its related species in order to expedite the programs being conducted for varietal improvement.

Approach: The associated problems are being tackled by application of the following approaches: dispersion of allozymes by norizontal starch-gel electrophoresis; isolateion mechanisms by means of controlled hybridizations, embryo culture, gamete fertility assays, meiotic chromosome behavior, modified segregations, and restricted recombination; male sterility by screening devices to identify spontaneous and induced mutants; mutants evaluated for male and female sterility, morphology, and stability of expression.

Progress: 82/04 to 88/09. Tomato male sterility: 1) more precise positioning of ms-15(superscript 47) on chromosome 2 to explore feasibility of selection via linked markers; 2) introgression of the useful ms-48 into 8 priority genetic and breeding stocks. L. esculentum - S. lycopersicoides hybrids: diploid recombinants in consecutive backcrosses. Twelve dominant and one recessive

monogenic traits thus introgressed from the wild parent segregated distinctly and some (Lac) express more intensely in L. e. background than in LS and LLS hybrids. Linkage inter se and with L. e. markers confirm the monogenic nature of the alien characters. Lac and Dls are tightly linked with Sp and b on 6L; Wa was positioned close to dl at ca. 31 on 8L. Deviations from expected BC ratios tend to favor alleles of the recurrent parent, sometimes highly significantly so. CMR participated in an IBPGR-sponsored trip to regions 2-4 (Antofagasta to Los Vilos), Chile in Marh to complete surverys and collection of wild tomato spp. in that country. New collections were made of L. chilense, the habitats noteworthy for their aridity and extreme alkalinity. TGSC activities: For the 6 month period (Jan. to June) 41 items, mostly genetic marker stocks, were accessioned; ca. 400 lots were planted for stock increase; seeds of 80 accessions were sent to NSSL for long-term storage; a revised list of miscellaneous genetic lines was issue in TGC 38; 1147 seed samples were distributed in response to 114 requests from 83 investigators.

Publications: 82/04 to 88/09
RICK, C. M. (1988). Molecular markers as aids for germplasm management and use in Lycopersicon. HortSci. 23:55-57. (presented at Symposium of XXII Int. Hort. Congr.).
RICK, C. M. (1988). Tomato-like nightshades: affinities, autecology, and breeders' opportunities. Econ. Bot. 42:145-154.
RICK, C. M. (1988). Evolution of mating systems in cultivated plants. pp. 133-147. In: Plant Evolutionary Biology. L. D. Gottlieb & S. K. Jain, eds. Chapman & Hall, London.

12.015 CRISO096773 GENOME ORGANIZATION IN THE CULTIVATED AND WILD SPECIES OF TOMATO

YODER J I; Vegetable Crops; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-VCR-4580-H Project Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 90

Objectives: To determine the molecular arrangement of genes and interspersing sequences in the cultivated and wild tomato species. To determine how genome organization correlates with a plant's tolerance to environmental and biological stress conditions. The molecular interaction of genes following interspecific hybridization will be determined to evaluate the biochemistry of aberrant phenotypes previously described genetically.

Approach: Techniques in molecular biology will be used to clone genes from both cultivated and wild tomatoes. Nucleotide sequencing will be used to compare similar genes from plants with aberrant phenotypes. Nucleic acid hybridization will be used to compare genome organization in different species as well as in plants collected from different environmental conditions.

Progress: 88/01 to 88/12. In order to initiate experiments directed towards comparing gene structure in cultivated and wild tomato species. We are isolating genomic sequences which encode enzymes active in anthocyanin biosynthesis. We have isolated cDNA clones for a key enzyme in flavonoid biosynthesis, chalcone synthase, from the tomato cultivar VF36. Nucleotide sequence analysis together with hybridization analysis indicate at least two genes actively express chalcone synthase in tomato seedlings. We have mapped two actively transcribed genes to different chromosomes using restriction fragment length polymorphism mapping. We are continuing this analysis to determine the total number of chalcone synthase genes present, their organization, and the extent of sequence divergence in distantly related lines.

Publications: 88/01 to 88/12
RICK, C. M. and YODER, J. I. (1988).
Classical and molecular genetics of tomato highlights and perspectives. Ann. Rev. Genet. 22:281-300.
O'NEILL, S. and YODER, J. I. Cloning and analysis of two chalcone synthase cDNAs from tomato. Submitted.

12.016 CRISO136115 TRANSPOSON MUTAGENESIS IN TOMATO

YODER J I; Vegetable Crops; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-VCR-5002-CG Project Type: CRGD Agency ID: CRGO Period: 01 JUL 88 to 30 JUN 90

Objectives: PROJ. 8800598. Isolate pollen specific gene promoters. Prepare chimeric transposon containing a pollen specific promoter and a maize transposase. Transform tomato plants to evaluate efficiency of pollen specific activity.

Approach: A cDNA library is prepared from tomato pollen. Differential hybridization with seedling mRNA is used to identify pollen abundant transcripts. The pollen specific transcripts are used to isolate the corresponding genes from a genomic library. S1 mapping and nucleotide sequencing is used to localize the pollen promoter regions. Pollen promoter is ligated to transpose coding region of the maize transposon Ac. Agrobacterium transformation used to introduce chimeric transposon into tomato cultivars.

Progress: 88/01 to 88/12. We are using maize transposable elements as biological mutagens in tomato ssp. In order to understand the mechanisms which control transposon activity in plants, we have made a number of in vitro alterations to the maize transposon AC and introduced these variants into tomato by assexual gene transfer methods. Transpositional activity of the altered elements is assessed in transgenic tomato plants by both biochemical and biological assays. We have known that certain internal deletions of Ac eliminate its capacity for autonomous transposition as well as reduce its transpositional activity when an intact element is present in trans. This work

is valuable in identifying molecular mechanisms of transposition, and hence provides the foundation for mutagenesis studies.

Publications: 88/01 to 88/12

No publications reported this period.

12.017 CRISO099645 TOWARDS A TRANSPOSON TAGGING SYSTEM IN TOMATO

YODER J K; Vegetable Crops; University of California, Davis, **CALIFORNIA** 95616.

Proj. No.: CA-D*-VCR-4721-CG Project Type: CRGD Agency ID: CRGO Period: O1 SEP 86 to 31 AUG 88

Objectives: PROJ 8600016. To develop a transposon tagging system in Lycopersicon so that gene coding for unknown products can be cloned.

Approach: To transform L. esculentum cultivars with the maize transposon and the Drosophila P element using Agrobacterium vectors. The copy number an apparent mobilization of the transposon will be determined in regenerated plants by nucleic acid hybridization. Plants in which the transposons have appeared to mobilize will be crossed to known homozygous, recessive, anthocyanin-less testers and the progeny screened for anthocyanin expression. The anthocyanin mutation frequency will be used to estimate the genetic potential of the transposon for mutagenesis.

Progress: 86/01 to 88/12. With the goal of developing a transposon mutagenesis system in tomato, we are examining the behavior of maize transposable elements which have been transformed into tomato. We have used both the autonomous Ac element as well as non-autonomous Ds elements as donor DNA. Ac is mobilized in transgenic tomatoes and we have shown the same element is capable of continually jumping for at least three generations. In contrast, Ds elements are stable in transformants. In F(subscript 1) hybrids between Ac and Ds transgenics, the Ds is activated and transposes to new positions. By reconstructing the maize Ac - Ds transposon system in tomato, we will be able to identify preferred integration sites of transposed elements as well as be able to use reverse genetics to dissect cis sequences required for transposition.

Publications: 86/01 to 88/12

YODER, J. I., PALYS, K., ALPERT, K. and LASSNER, M. (1988). Ac transposition in transgenic tomato plants. Mol. Gen. Genet. 213:291-296.

JACOBS, J. P. and YDDER, J. I. (1988). Ploidy levels in transgenic tomato plants rapidly determined by chloroplast number. Plant Cell Rep. In press.

LASSNER, M. W., PALYS, J. M. and YODER, J. I. Genetic transactivation of dissociator elements in transgenic tomato plants. Submitted.

12.018* CRISO087835 BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA

COOKSEY D A; Plant Pathology; University of California, Riverside, **CALIFORNIA** 92521. Proj. No.: CA-R*-PPA-4259 Project Type: STATE Agency ID: SAES Period: O1 JUN 82 to 11 AUG 88

Objectives: To study mechanisms of biological control of crown gall disease and mechanisms of resistance to agrocin 84. To study interactions of bacterial pathogens with other microorganisms and develop new agents for biological control.

Approach: Investigate binding of biological control agents and mutants of these agents to plant tissue culture suspension cells. Restriction endonuclease analysisof agrocin resistant mutants. Genetic manipulations for the development of new biological control agents.

Progress: 82/06 to 88/08. A novel integrated biological/chemical control method was developed for bacterial speck disease of tomato using genetically engineered nonpathogenic, copper-resistant mutants of Pseudomonas syringae pv. tomato. The nonpathogenic mutants colonized tomato plants epiphytically and were used to exclude pathogenic strains. Since the mutants were copper resistant, they could be combined with copper bactericide treatments to provide a greater control than obtained with the mutant alone or the bactericide alone. Resistance to copper was shown to be common in strains of P. syringae pv. tomato from California. Resistance was determined by a conserved plasmid, and a physical map of the plasmid was constructed. Copper resistance genes were cloned from this plasmid and sequenced. Copper resistance was determined by four genes organized as an operon. The potential use of these genes in modifying various beneficial microbes for compatibility with copper bactericide applications is under investigation.

Publications: 82/06 to 88/08

COOKSEY, D.A. (1988). Reduction of infection by Pseudomonas syringae pv. tomato using a nonpathogenic, copper-resistant strain combined with a copper bactericide. Phytopathology. 78:601-603.

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MELLANO, M.A., and COOKSEY, D.A. (1988).

Nucleotide sequence and organization of copper resistance genes from Pseudomonas syringae pv. tomato. J. Bacteriol.

170:2879-2883.

12.019 CRISO135476 CHARACTERIZATION OF PATHOGENICITY AND DEVELOPMENT OF CONTROL STRATEGIES FOR PLANT PATHOGENIC BACTERI

COOKSEY D A; Plant Pathology; University of California, Riverside, **CALIFORNIA** 92521. Proj. No.: CA-R*-PPA-4969-H Project Type: HATCH Agency ID: CSRS Period: 12 AUG 88 to 30 SEP 92

Objectives: To characterize genes and gene products that determine pathogenicity and copper resistance in bacterial pathogens. To test and extend the concept of using nonpathogenic mutants of plant pathogenic bacteria for biological control of bacterial diseases.

Approach: Subcloning, transposon and deletion mutagenesis, restriction mapping, and nucleic acid sequencing will be used to physically characterize cloned genes involved in pathogenicity. Gene products associated with pathogenicity and copper resistance will be characterized by biochemical methods, and antibodies will be produced to these products for localization studies. Stable nonpathogenic deletion mutants with copper resistance genes integrated in the genome will be tested for biological control in combination with conventional copper bactericides.

Progress: 88/08 to 88/12. We previously cloned and sequenced a copper resistance gene cluster from a conserved plasmid in copper-resistant strains of Pseudomonas syringae pv. tomato. This plant pathogen is resistant to copper bactericides that are used commercially for disease control. Recent work on gene regulation in this system showed that the genes controlling copper resistance are inducible by copper. Analysis of mRNA demonstrated that regulation is at the transcriptional level. Using gene probes specific for each of the four genes of the cluster, mRNA levels for each gene were shown to be coordinately induced over time and with increasing concentrations of copper. A single, copper-inducible promoter was cloned from the region 5' to the first gene of the cluster, suggesting that the four genes controlling copper resistance are transcribed as an operon.

Publications: 88/08 to 88/12
MELLANO, M.A., and COOKSEY, D.A. (1988).
 Induction of the copper resistance operon
 from Pseudomonas syringae pv. tomato. J.
 Bacteriol. 170:4399-4401.

12.020 CRISO142046 MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS

JARRET R L; OKUBU C; Biology; Florida International University, Miami, FLORIDA 33101.

Proj. No.: 6607-21000-001-025

Project Type: COOPERATIVE AGREE. Agency ID: ARS Period: O1 MAR 87 to 31 DEC 89

Objectives: To map restriction endonuclease cleavage sites on the chloroplast genome ofsweet potato and related species. To evaluate genetic diversity in indigenous and newly introduced sweet potato germplasm using polymorphisms for these restriction sites. To identify the progenitor species of cultivated sweet potatoes using molecular markers.

Approach: Total (nuclear, chloroplast (cp) and mitochondrial) DNA will be isolated, digested and fragments separated on agarose gels.

Digests will be transferred to a solid support and probed with radioactively labelled cpDNA specific markers. A cpDNA map will be constructed. Variation between cp genomes from sweet potato and related species will be compared using currently available computer software for analysis of restriction fragment length polymorphisms.

Progress: 88/01 to 88/12. A detailed extraction protocol for isolation of chloroplast DNA (cpDNA) from I. batatas and related species has been developed. This technique has been used to isolate cpDNA from Ipomoea species batatas, triloba, cordatotriloba, ramosissima, lacunosa and trifida. Restriction endonuclease digests of cpDNA from these species has revealed numerous polymorphisms which are being analyzed to study the systemics of I. batatas and Ipomoea section Batatas species.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

12.021 CRISO074078 GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELD, PEST RESISTANCE AND FOOD VALUE

PATIL S S; Plant Pathology; University of Hawaii, Honolulu, **HAWAII** 96822.

Proj. No.: HAWO0741-H Project Type: HATCH Agency ID: CSRS Period: O1 OCT 84 to 30 SEP 89

Objectives: Determine and exploit genetic mechanisms regulating horizontal resistance.

Approach: To determine the genetic and biochemical basis of pathogenicity of Pseudomonas syringae pv. phaseolicola to phaseolus vulgaris L. two approaches will be taken. First, genomic libraries of strains of the pathogen which are avirulent to the bean cultivar Red Mexican will be made. Clones from these libraries will be mated individually or en masse with strains of P. s. pv. phaseolicola which are virulent to the same bean cultivar using a triparental mating procedure. Transconjugant will be tested on Red Mexican to determine if any of them have acquired the DNA fragments from library clones which encode the avirulence function.

Progress: 87/10 to 88/09. We previously reported isolation of a genomic clone from Race 1 strain (HB-33) of Pseudomonas syringae pv. phaseolicola that harbors an avirulence (avr) gene against cultivar Red Mexican of bean which has a single dominant resistance gene against Race 1 of the pathogen. This avr gene is expressed in strains of Race 2 when they are inoculated in Red Mexican. In further studies the genomic clone has been characterized by restriction endonuclease mapping and the location of the gene is determined by Tn5 mutagenesis. A Tn5 insertion within a Hind III-Bam H1 fragment of the insert neutralizes gene function. Further work on the characterization of the avr gene by Tn5 mutagenesis and marker exchange is in progress.

Publications: 87/10 to 88/09 No publications reported this period.

12.022 CRISO137864 CHARACTERIZATION OF THE MI LOCUS CONFERRING RESISTANCE TO MELOIDOGYNE INCOGNITA IN TOMATO

WHITE F F; Plant Pathology; Kansas State University, Manhattan, KANSAS 66506. Proj. No.: KAN00792 Project Type: HATCH Agency ID: CSRS Period: 01 JUL 89 to 30 SEP 92

Objectives: Analyze the restriction fragment length polymorphisms (RFLP) in the region of the mi locus to determine the extent of alien DNA introgression in tomato. Since mi is apparently linked to the locus for isozyme 1 of acid phosphatase (aps1), RFLP probes are sought for the aps1 locus. A test for nematode susceptibility on transgenic roots is to be established. The amount of alien DNA that can be transferred to roots will be analyzed. The role of superoxide dismutase in nematode resistance will be examined.

Approach: RFLP probes will be used to analyze the extent of Lycopersicum peruvianum DNA in nematode resistant tomato lines (Lycopersicum esaulentum). Additional probes are to be obtained from the chromosomal region of the mi gene. Probes will be prepared and used in laboratory techniques including DNA isolation, northern and Southern blot hybridization analyses, protein purification, and DNA cloning. Plant testing will involve inoculation with nematodes and Agrobacterium-mediaseedplant transformation.

12.023 CRIS0099069 RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS

HDUTZ R L; Horticulture & Landscape Architecture; University of Kentucky, Lexington, KENTUCKY 40506.

Proj. No.: KY00586 Project Type: HATCH Agency ID: CSRS Period: 01 DCT 86 to 30 SEP 89

Objectives: Understand the mechanism(s) of action of factors affecting vegetable yield.

Approach: Study the effect of light intensity and CD(2) concentration on steady-state photosynthetic CO(2) assimilation and ribulose -1. 5-bisphosphate carboxylase/oxygenase activity. Quantity light-dependent and CD(2)-dependent levels of oxidative and reductive photosynthetic carbon cycle intermediates.

Progress: 88/01 to 88/12. Two adjacent N-terminal tryptic peptides of the large subunit of ribulose bisphosphate carboxylase/oxygenase ?3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39? from spinach, wheat, tobacco, and muskmelon were removed by limited tryptic proteolysis. Characterization by peptide sequencing, amino

acid composition, and tandem mass spectrometry revealed that the N-terminal residue from the large subunit of the enzyme from each plant species was acetylated proline. However, the penultimate N-terminal peptide from the large subunit of both the tobacco and muskmelon enzyme, while identical, differed from the corresponding peptide from spinach and wheat by containing a trimethyllysyl residue at position 14. A comparison of the DNA sequences for the large subunit of ribulose bisphosphate carboxylase/oxygenase indicate that the N-terminus has been post-translationally processed by removal of methionine-1 and serine-2 followed by acetylation of proline-3. In addition, for the enzyme from tobacco and muskmelon a third post-translational modification occurs at lysine-14 in the form of (sigma)-N-trimethylation. Limited tryptic proteolysis of ribulose bisphosphate carboxylase/oxygenase (ribulose-P carboxylase) resulted in the ordered release of two adjacent N-terminal peptides from the(superscript 2) large subunit and irreversible catalytic inactivation

Publications: 88/01 to 88/12 ARCHBDLD, D.D. and HDUTZ, R.L. (1988). Photosynthetic characteristics of strawberry plants treated with paclobutrazol or flurprimidol. HortSci. 23:200-202.

NABLE, R.D., HDUTZ, R.L. and CHENIAE, G.M. (1988). Developing Mn toxicity in young tobacco leaves. I. Early inhibition of photosynthesis during development of Mn toxicity in tobacco. Plant Physiol. 86:1136-1142.

HDUTZ, R.L., NABLE, R.O. and CHENIAE, G.M. (1988). Developing Mn toxicity in young tobacco leaves. II. Evidence for effects on the in vivo activity of ribulose-bisphosphate carboxylase/ oxygenase during development of Mn toxicity in tobacc.

MULLIGAN, R.M., HOUTZ, R.L. and TDLBERT, N.E. (1988). Reaction-intermediate analogue binding by ribulose bisphosphate carboxylase/oxygenase causes specific changes in proteolytic sensitivity. Proc. Natl. Acad. Sci. USA 85:1513-1517.

BIERNBAUM, J.A., HDUTZ, R.L. and RIES, S.K. (1988). Field studies with crops treated with colloidally dispersed triacontanol. J. Amer. Soc. Hort. Sci. 113:679-684.

CRISO049444 12.024 GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS

SCHAEFFER G W; CRISS; MATHEWS; Agricultural Research Service; Beltsville Agr Res Center, Beltsville, MARYLAND 20705. Proj. No.: 1209-20173-003-00D

Project Type: INHDUSE Period: 01 OCT 84 to 12 AUG 86 Agency ID: ARS

Objectives: Terminate 1209-20173-002, accession 0043396. Start 1209-20173-003 with \$300,000 net to bench funds planned for 1209-20173-002 and \$50,000 from \$4.1 million administrative reduction funds. G. Still is NPS contact. Table 1 - 11F.

Approach: Twelve on-going projects will receive additional funding to accelerate research on genetic engineering, gene mapping and transfer, hormonal regulation, membrane structure, and other biotechnologies for improved cro p productivity, including control of insects, diseases, and other pests. This high-technology research will improve the fundamental understanding of important agricultural problems and lead to innovative solutions to agricultural problems.

Progress: 84/01 to 84/12. The 160-kilobase chloroplast (ct) DNA of Daucus carota was cloned, and a physical map was constructed. The circular ctDNA map delineates the positions of an inverted repeated region and genes encoding ribosomal RNAs, the large subunit of ribulose biphosphate carboxylase and the 32-kilodalton protein. The ctDNA of a wild species, D. pusillus was also mapped. Although it did show a great deal of variation in restriction endonuclease digestion patterns, the gene positions were found to be conserved. The D. carota 450-kilobase mitochondrial genome was cloned in a bacteriophage lambda vector, and portions of this gene library were partially mapped to the complete circular genome. The ribosomal RNAs and the gene encoding the protontranslocating subunit of the mitochondrial ATPase were localized and mapped. Analysis of flanking regions reveals that these genes are found in only one copy per mitochondrial genome. Changes in gene expression in develoing somatic embryos of D. carota were detected by two-dimensional gel electrophoresis of nascent proteins. Some changes were observed as early as one day föllowing induction of the in vitro developmental sequence by removal of auxin from the culture medium. Putative mutants of D. carota, temperature-sensitive for somatic embryo development, were isolated by a filtration enrichment protocol.

Publications: 84/01 to 84/12
DE BONTE, L.R., MATTHEWS, B.F., and WILSON,
K.G. 1984. Variation in plastid and
mitochondrial DNAs in the genus Daucus.

Amer. J. Bot. 7:932-940.

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MATTHEWS, B.F., DE BONTE, L.R. 1985. Chloroplast and mitochondrial DNAs of the carrot and its wild relatives. Plant Molec. Biol. Reporter. (In press).

MATTHEWS, B.F. and WIDHOLM, J.M. 1985.
Organelle DNA compositions and isoenzyme expression in an interspecific hybrid of Daucus. Molec. Gen. Genet. (In press).

DIENER, T.O., OWENS, R.A., and CRESS, D.E. 1984. Plant viroids: new diagnostic methods...agriculture. In: Control of Virus Diseases, E. Kurstak and R.G.

Marusyk, eds., Dekker, New York, pp. 345-360. OWENS, R.A., KIEFER, M.C., and CRESS, D.E. 1985. Biological activity of cloned ...cDNAs. In: Subviral Pathogens of Plants and Animals, Maramorosch and McKelvey, eds., Academic Press, NY. (In press).

HAMMOND, R.W., KIEFER, M.C., CRESS, D.E. and OWENS, R.A. 1984. Probing viroid structure-function...cDNAs. In: Molec. Form and Function of Plant Genome, Plenum, NY. (In press).

12.025 CRISO132316 CLONING AND EXPRESSION OF GENES INVOLVED IN AMINO ACID BIOSYNTHESIS

MATTHEWS B F; Plant Molecular Genetics; Rm 101 Bldg 008, Beltsville, MARYLAND 20705. Proj. No.: MDR-8700602 Project Type: CRG0 Agency ID: CRG0 Period: 01 SEP 87 to 31 AUG 90

Objectives: The objectives are to sequence the gene(s) encoding homoserine dehydrogenase and compare the sequence to other gene sequences, complete the characterization of the physical & kinetic properties of homoserine dehydrogenase, purify aspartokinase to homogeneity for characterization and if time permits, isolate the gene(s) encoding aspartokinase for mapping, sequencing, and comparison to other known gene sequences. PROJ. 8700602.

Approach: This laboratory has constructed a cDNA expression library in lambdagt11 from carrot mRNA. We have purified homoserine dehydrogenase to homogeneity and have generated antibody against it. We are currently screening the library with the antibody to identify clones containing the gene encoding homoserine dehydrogenase. Furthermore, the amino acid sequence of the enzyme will be determined by micro-sequencing. The amino acid sequence will be compared to the DNA sequence to confirm the gene's identity. A similar approach will be used for the cloning and identification of the genes encoding aspartokinase.

Progress: 87/09 to 88/09. Homoseine dehydrogenase (HSDH) was purified to homogeneity from carrot and characterized (Paper submitted to JBC Nov. 1988). The enzyme is a polymer with a subunit size of 85,000. Antibody to native H\$DH was raised in three mice. The antibody was specific for carrot HSDH on Western blots and immunoprecipitated active enzyme (paper in prep.). The enzyme cross reacted with soybean HSDH, but did not react with denatured carrot HSDH. Two lambda gtll cDNA libraries were screened, an amplified and an unamplified library. No positive plaques were identified. HSDH denatured by heat and SDS treatment has been inoculated into a rabbit to raise new antibody specific to epitopes present in the denatured form of HSDH, presumably synthesized in E. coli. Aspartokinase has been purified over 100 fold. Two other genes have been identified tentatively as asparagine synthetase and aspartate amino transferase (AAT). AAT has a molecular weight of 105,000 and is a dimer. There may be two very similar isoenzymic forms of AAT in carrot.

Publications: 87/09 to 88/09

MATTHEWS, B.F., REARDON, E.M., TURANO, F.J. and WILSON, B.J. 1988. Amino acid biosynthesis in plants: an understanding at the molecular level. Plant Molecular Biology Reporter 6:137-154.

MATTHEWS, B.F. and FARRAR, M.J. 1988.

MATTHEWS, B.F. and FARRAR, M.J. 1988.
Purification and characterization of
homoserine dehydrogenase from Daucus carota
cell suspension cultures. Genome 30:456.
(Abstract).

TURANO, F.J., JORDAN, R.L. and MATTHEWS, B.F. 1988. Verification of a homoserine dehydrogenase-specific polyclonal antibody.

Genome 30:456. (Abstract).

MATTHEWS, B.F. and FARRAR, M.J. 1988.

Purification and interconversion of isoenzymic forms of homoserine dehydrogenase from all suspension cultures of Daucus carota. Mid Atlantic Plant Molecular Biology Society, p. 35.(Abs.).

TURANO, F.J., JORDAN, R.L. and MATTHEWS, B.F. 1988. Verification of a homoserine dehydrogenase specific monoclonal antibody. Mid Atlantic Plant Molecular Biology Society, p.41. (Abstract).

12.026 CRISO136072 MOLECULAR MARKERS FOR BRASSICA CAMPESTRIS CHROMOSOMES

BERNATZKY R; Plant & Soil Sciences; University of Massachusetts, Amherst, MASSACHUSETTS 01003.

Proj. No.: MASOO645 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 88 to 30 SEP 93

Objectives: The objective of the proposed study is to develop a genetic linkage map in an important crop plant, Brassica campestris, using molecular markers. Ultimately, the map of B. campestris chromosomes will be compared to a map in B. napus, an oil seed crop, using the same set of markers. B. napus is a tetraploid derived from B. campestris and B. oleracea and these markers will help to simplify the complex inheritance in this species. The markers will also be useful as gene tags in plant improvement programs of both crops.

Approach: The markers will consist of DNA coding sequences that will be prepared from mRNA of B. campestris. These sequences will be visualized in a segregating population of B. campestris through restriction fragment length polymorphisms (RFLP analysis). Standard genetic analysis will be employed to determine the linkage relationships among markers. A subset of single or low copy sequences will be similarly used on a segregating population of B. napus. In addition, a set of recombinant inbred (RI) lines will be developed from a cross between diverse accessions of B. campestris. The RI lines will provide a permanent source of materials for mapping and will be available to other researchers.

12.027 CRISO134841 APPLICATION OF MOLECULAR GENETIC APPROACHES TO VEGETABLE CROP IMPROVEMENT

GRUMET R; Horticulture; Michigan State
University, East Lansing, **MICHIGAN** 48824.
Proj. No.: MICLO1549 Project Type: HATCH
Agency ID: CSRS Period: O3 JUN 88 to O2 JUN 93

Objectives: To apply molecular genetic approaches to vegetable crop improvement.

Approach: A variety of components are necessary to achieve this goal. These include: Establish molecular genetic technologies that are

applicable to vegetable crop species by developing regeneration and transformation strategies. Primary emphasis will be on Agrobacterium-mediated transformation systems. Crops of importance to Michigan that will receive initial effort are cucurbit crops (cucumbers, squashes, melons) and celery. Identify and clone genes with potential usefulness for crop improvement. A focus will be on disease resistance genes. Specific targets include viral-derived resistance genes and host-derived pathogenesis related proteins. Introduce genes into the crop plant by engineering cloned genes into a functional expression vector, transforming the plant, verifying expression, testing for the desired trait, and determining if it is stably inherited. Increase the background knowledge of the genetics of vegetable crops by using both conventional (e.g. determinations of heritability, numbers of genes, dominance/recessive behavior) and molecular (e.g.

Progress: 88/06 to 88/12. The major focus to this program is the application of molecular genetic approaches to vegetable crop improvement. Zucchini yellows mosaic virus (ZYMV) infection of cucurbits is one economically important disease for which it may be possible to genetically engineer resistance. Toward this end we have made the following progress in the past year: Purified ZYMV virions from infected zucchini plants, isolated the viral RNA, made double stranded cDNA copies of the genome, and cloned into the Bluescript vector; Verified that we have a nested set of 25 clones beginning at the 3' end and ranging in size from 0.3-6.0 Kb (thus 50-70% of the genome has been cloned); began constructing a restriction map. Located the coat protein gene and began DNA sequencing; data show that the translated amino acid sequence of this gene shares a highly conserved region with other related viral coat proteins. Made polyclonal antibodies to ZYMV coat protein for use in ELISA tests. Set up an Agrobacterium tumefaciens - mediated transformation system muskmelon. Preliminary data from regenerated melon plants indicates successful transfer of the NPT marker gene. Studies are also in progress to increase regeneration efficiency of cucumber. Began developing a transient assay system for use in testing new gene constructs using electroporated cucumber protoplasts. Two conventional breeding/genetics projects were also initiated.

Publications: 88/06 to 88/12
GRUMET, R. and ZAGULA-HOEFLER, K. 1988.
Purification and cloning of zucchini
yellows mosaic virus. Hort. Science
23:99(Abstract).

12.028 CRISO134748
MANIPULATION AND TRANSFER OF NUCLEAR AND
ORGANELLE GENES IN VEGETABLE CULTIVARS BY CELL
FUSION

SINK K; Horticulture; Michigan State University, East Lansing, **MICHIGAN** 48824. Proj. No.: MICLO1544 Project Type: HATCH Agency IO: CSRS Period: 23 MAY 88 to 22 MAY 93

Objectives: Oevelop and apply asymmetric nuclear gene transfer for the improvement of vegetable cultivars. Oevelop cell fusion systems for the manipulation of plastome and mitochondrial genomes in vegetable cultivars. Develop direct DNA transformmation techniques targeting plant protoplasts.

Approach: Agrobacterium transformed donor vegetable species are produced. The T-ONA inserts are mapped, donor lines irradiated and fused with recipient protoplasts. Transmission of organelles is done by altering their status in donor-recipient protoplasts by irradiation and/or chemical inhibitors. Isolated ONA is electroporated into protoplasts. Gene transfers in all cases are monitored by molecular means and their expression in regenerated whole plants.

Progress: 88/05 to 88/12. Leaf sections of Solanum melongena L. (cv. Black Beauty) were Leaf sections of inoculated with disarmed Agrobacterium strains 715, pCIB10 and pMON200. Eggplants resistant to kanamycin were only obtained with the latter strain. Slot-blot ayalysis indicated that 3 plants had the NOS-NPT sequences homologous to those of the vector; they produced nopaline and exhibited kanamycin resistance for a 6-month period. RFLP analysis of Lycopersicon esculentum (LE) + Solanum lycopersicoides (SL) somatic hybrids indicated biased transmission of organelles. The plastids in 68/70 plants, each regenerated from a separate callus, were from LE. One plant, 240, had SL and another, 63, had a mixture of plastids. Forty-six of the same 70 plants had mtONA of SL including plant 240. One plant had novel mtONA fragments which may have resulted from recombination or rearrangement. RFLP's were also used to identify and quantitate the nuclear contributions of LE and SL to the hybrids. Single copy clones 2-13, 2-17, 3-288, and pHA2 (45s ribosomal); all mapped to LE chromosome 2, were used as probes on 47 somatic hybrids. There were more than two SL copies in most hybrids; one hybrid had lost at least one LE copy. Most variation in single copy number was consistant with the presumed changes in chromosomes occurring in the protoplast donor cell suspension of SL. In asparagus breeding, seed was collected from three crosses of M.S.U. females \times NJ22-8.

Publications: 88/05 to 88/12

FORO-LOGAN, J. and SINK, K.C. 1988. Plant regeneration from protoplasts of Petunia alpicola. Hort. Science 23:393-395.

GURI, A. and SINK, K.C. 1988. Agrobacterium transformation of eggplant. J. Plt. Physiol. 133:52-55.

GURI, A. and SINK, K.C. 1988. Interspecific somatic hybrid plants between eggplant (Solanum melongena) and Solanum torvum. Theor. Appl. Genet. 76:490-496.

GURI, A., LEVI, A. and SINK, K.C. 1988.
Somatic hybrid plants between Lycopersicon esculentum and Solanum nigrum. Gen. Genet. 212:191-198.

GURI, A. and SINK, K.C. 1988. Organelle composition in somatic hybrids between as atrazine resistant biotype of Solanum

nigrum and Solanum melongena. Plt. Sci. 58:51-58.

LEVI, A., RIOLEY, B.L., and SINK, K.C. 1988. Biased organelle transmission in somatic hybrids of Lycopersicon esculentum and Solanum lycopersicoides. Curr. Genet. 14:177-182.

MOORE, P.P. and SINK, K.C. 1988.

Characterization of a Lycopersicon esculentum + Solanum lycopersicoides somatic hybrid plant lacking a glutamate oxaloacetate transaminase isozyme. Plant Cell, Tissue and Organ Culture 13:39-47.

12.029 CRISO132374 T-DNA: A NOVEL MARKER SYSTEM FOR ASYMMETRIC GENE TRANSFER BY CELL FUSION

SINK K C; Horticulture; Michigan State
University, East Lansing, MICHIGAN 48824.
Proj. No.: MICLO8045 Project Type: CRG0
Agency IO: CRG0 Period: 15 SEP 87 to 30 SEP 90

Objectives: PROJ. 8700333. A modified Agrobacterium T-ONA sequence will be used as a random marker of the donor nuclear genes that concomitantly serves as a selectable marker for detecting transfer at the cell level. Using tomato species as a model system the objectives are: Demonstrate the transfer of a mapped T-ONA insert and linked genes into a recipient genome via cell fusion; Determine the relationship between irradiation level(s) on the T-ONA insert donor and the size, mode of integration and stability of the transferred genes; Establish the mode of inheritance of the transferred T-ONA and linked genes.

Approach: The scientific approach will be to use genetically mapped T-ONA inserts in an interspecific tomato hybrid genome having known linkage associations to isozymes, cONA markers and morphological loci, the "tagged genes" as the cell donor. Donor protoplasts, which have been irradiated to fragment the nuclear genome, will be fused with an efficient plant regenerating wild tomato species as the recipient. This recipient also contrasts genetically to the donor genotype; thus, permitting biochemical and inheritance studies to confirm alien gene transfer. Such experiments using tomato as a model system should lead to a generalized asymmetric marker gene transformation system for the interchange of desired traits in crop plants.

Progress: 88/01 to 88/12. Two lines (L-54 and L-181) of the Agrobacterium transformed tomato x Lycopersicon pennellii sexual hybrid in which the T-ONA, sequence containing the chimeric gene NOS-NPT conferring kanamycin resistance, were inserted into tomato chromosomes 12 and 2 respectively, and line LA 1990 of the species Solanum lycopersicoides were fused in this study. Leaf protoplasts of L-54 and L-181 (donors) were gamma-irradiated at O (control), 50, 100, and 200 Gy and immediately fused with S. lycopersicoides (recipient) as callus-derived protoplasts using PEG and OMSO to produce symmetric and asymmetric hybrids. Recently, symmetric somatic hybrids between non-irradiated L-54 and LA 1990

were verified by isozyme analysis. From the fusion of LA 1990 protoplasts with L-54 gamma-irradiated protoplasts, approximately 35 kanamycin resistant plants were obtained that are morphologically similar to LA 1990, the recepient parent. These plants may be the desired asymmetric somatic hybrids. At the present time the putative symmetric and asymmetric somatic hybrids are being propagated in vitro prior to transfer to the greenhouse. Parallel, proto-calluses derived from fusions between LA 1990 and gama-irradiated L-181 protoplasts are placed on regeneration medium supplemented with kanamycin. In another study, metaphase chromosomes were isolated from the Agrobacterium transformed tomato x L. pennelli, line 295.

Publications: 88/01 to 88/12

NO PUBLICATIONS REPORTED THIS PERIOD.

12.030 CRISO131860 CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIDS AND CYBRIDS OF TOMATO

O'CONNELL M A; Agronomy & Horticulture; New Mexico State University, Las Cruces, **NEW MEXICO** 88003

Proj. No.: NM-1-5-28237 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 87 to 31 AUG 90

Objectives: 8700329 1)-Construction of a collection of tomato lines each with a unique and novel cytoplasmic genome. 2)-Construction of asymmetric fertile somatic hybrids of tomato.

Approach: 1)-Cytoplasts will be prepared from suspension cell cultures of wild relatives of tomato, and fused, using polyethylene glycol, to iodoacetate treated protoplasts prepared from tomato cultivars. The protoplasts will be cultured and regenerated to whole plants. The regenerants will be characterized using isozyme polymorphisms, plant morphology, plant fertility, and restriction maps of organellar genomes. 2)-Protoplasts prepared from suspension cell cultures of "Lycopersicon pennellii" will be exposed to varying doses of gamma radiation, 10 to 100Krads, and then fused with untreated protoplasts prepared from cultivars of tomato. Fused protoplasts will be selected using a fluorescence activated cell sorter. Regenerated plants will be characterized with isozymes and cONA markers. The extent of introgression of the irradiated genome in the fusion product will be determined by comparing the molecular markers present in the regenerant with the map location of the marker. The regenerants will be characterized as in 1).

Progress: 88/01 to 88/12. Asymmetric somatic hybrids generated by fusing irradiated protoplasts of Lycopersicon pennellii with untreated protoplasts of cultivated tomato, L. esculentum, are being constructed and characterized. Using cONA clones and described RFLPs in tomato, we have been able to demonstrate that two asymmetric hybrids which are clonally related, have lost either both homologues of chromosome 12 from tomato or both

homologues of chromosome 1 and 12 from tomato. Construction of cybrids between tomato cultivar UC82 and wild species, L. peruvianum, L. pennellii, L. chilense and Petunia hybrida are underway. These constructions were attempted using either physical isolation of cytoplasts from protoplasts of the indicated species, or exposure to 100 kRads of (superscript 60)Co. Constructions of asymmetric somatic hybrids are in culture following fusion of of L. pennellii protoplasts exposed to 3, 6, 9, 15, 21 or 100 kRads of (superscript 60)Co with tomato protoplasts prepared from cultivars UC82 or Cal-Ace. Characterization of the mitochondrial genome in some of the regenerated somatic hybrids is underway. Cosmid clones carrying mapped regions of the tomato mitochondrial genome have been used as probes to assess the extent of recombination in the mitochondrial genome of somatic hybrids between tomato cultivar UC82 and L. pennellii.

Publications: 88/01 to 88/12

No publications reported this period.

12.031 CRISO133477 SOMATIC CELL GENETICS IN VEGETABLE CROPS

O'CONNELL M A; Agronomy & Horticulture; New Mexico State University, Las Cruces, **NEW MEXICO** 88003.

Proj. No.: NM-1-5-27411 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: 1) Construct and characterize cybrids, asymmetric and complete somatic hybrids of tomato. 2) Evaluate feasibility of objective 1 with other vegetable crops, onion and pepper. 3) Characterize mitochondrial genome of tomato. 4) Characterize heat shock response in heat sensitive and resistant lines of cotton. 5) Generate and utilize RFLP maps in vegetable crops.

Approach: 1) Protoplasts and cytoplasts will be prepared from appropriate explants of cultivars of tomato and from wild relatives of tomato. The protoplasts or cytoplasts will be fused, and the products regenerated into plants. The regenerants will be characterized using nuclear genome and organellar genome RFLPs, isozyme activities, and morphology and fertility. 2) Transfer of protoplast technology to the recalcitrant crops, onion and pepper will be tried. 3) Libraries containing tomato mitochondrial ONA have been prepared. The recombinant clones will be mapped with restriction enzymes, and a physical map of the genome constructed. Genes will be located on the map using heterologous probes and Northern analysis. 4) The heat shock proteins synthesized by heat tolerant and sensitive lines will be determined by in vivo protein synthesis experiments and analysis of the radioactive prOteins on 2-dimensional gels.

Progress: 87/09 to 88/12. The chloroplast ONA of tomato cultivar Cal Ace was used to generate a library of the Sal 1 fragments of the genome. These cloned fragments are now being used to map the chloroplast genome of pepper, Capsicum annuum. In addition, the

chloroplast ONA from several species of Capsicum have been purified and analyzed on agarose gels. These results will allow us to generate a taxonomic relationship between the species based on RFLPs in the chloroplast genome. Comparisons were made between the expression of head shock proteins (HSPs) in genetically characterized heat tolerant and heat sensitive lines of cotton. These comparisons were based on electrophoretic analysis of in vivo labelled proteins. Several HSPs were identified on two dimensional gels which were expressed uniquely in either the tolerant (26 kDa) or sensitive cotton line (24 kOa and 18 kOa). However, the HSP pattern displayed in a heat tolerant BC-3 individual was that of the heat sensitive parent. The expression of HSPs in two species of tomato, Lycopersicon esculentum and L. pennellii, and the interspecific hybrid were characterized. Unlike the results obtained in the cotton studies, major differences in the sizes of the classes of HSPs expressed by these sexually compatible species were observed.

Publications: 87/09 to 88/12
No publications reported this period.

12.032 CRISO033955 EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH

TANKSLEY S D; Horticulture; New Mexico State University, Las Cruces, **NEW MEXICO** 88003. Proj. No.: NM-1-5-28223 Project Type: CRGO Agency IO: CRGO Period: O1 SEP 82 to 31 OEC 85

Objectives: Proj. No. 8200119. The utility of mapped, multiple isozyme markers in tomato will be tested in: mapping and tagging male-sterile genes, determining the number and map position of major genes responsible for low-temperature seed germination derived from high-altitude ecotypes of L. hirstum and L. chilense, and to facilitate the transfer of those genes into tomato breeding lines, testing for and potentially characterizing natural and induced somatic recombination and chromosome elimination in cultured cells and tissues of sterile intergeneric hybrids.

Approach: Enzyme electophoesis utilizing starch and polyacrylamide will be used to screen for rare-allele varieties in tomato species germplasm. Rare alleles will be concentrated into marker stocks by inter-crossing. Stocks will be used to achieve above described objectives.

Progress: 82/09 to 85/12. Tomato. A multiple isozyme stock has been produced in the homozygous state. This stock carries rare alleles at enzyme loci strategically located throughout the genome. The stock is useful for locating and tagging monogenic traits as well as for analysis of quantitatively-inherited traits via multi-locus analysis. Two nuclear male-sterile genes (ms-10 and ms-32) have been associated with rare-alleles at tightly-linked enzyme-coding genes, providing a convenient selection method for interline transfer of nuclear male-sterility. The gene for

self-incompatibility has been located in L. peruvianum, using enzyme markers as genetic probes. This S locus resides on chromosome 1. Pepper (Capsicum annuum). A number of isozyme loci have been mapped in the pepper genome and used to dissect the genetics of the multiple flower character found in C. chinense. Onion (Allium cepa). Isozyme are being used to monitor introgression of A. fistulosum chromosomes in A. cepa via alien addition lines. The data indicate this approach may allow development of A. cepa cultivars carrying pink root resistant from A. fistulosum. This research resulted in 11 publications, 2 M.S. and 2 Ph.D. degrees. Three post-doctoral appointments supported this research.

Publications: 82/09 to 85/12 NO PUBLICATIONS REPORTED THIS PERIOD.

12.033 CRISO133189 MOLECULAR ANALYSIS OF THE EFFECTS OF THE ALC MUTANT IN TOMATO RIPENING

MUTSCHLER M A; Plant Breeding; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-149307 Project Type: CRGO Agency IO: CRGO Period: O1 SEP 87 to 28 FEB 90

Objectives: Proj. 8701115. The short and moderate term goals of this proposal are to determine what effects alc has on the levels of mRNA's homologous to ripening-specific clones, whether these effects of alc are due to changes in transcription or the degradation of the mRNAs of the affected loci, how the effects of alc on levels of specific mRNA are mediated, and to determine the number and location of functioning loci homologous to the ripening specific genes. The effects of fruit detachment on the levels of ripening specific m-RNAs will also be studied. A long term goal, which may come after the term of this proposal, is to clone & characterize the alc locus and its gene product, and to determine the nature of the reversion event which produced Alcobaca-red.

Approach: The techniques used to obtain the short to moderate term goals include standard molecular tools (Southern and Northern gels and slot blots), RFLP mapping & nuclear runoff. The methods used to obtain the long term goal depends on identification of a method of cloning what may be a regulatory gene. The results of the short to moderate term studies will provide the information needed to make this decision.

Progress: 88/01 to 88/12. The alc mutation affects the ripening&storability of tomatoes. Polygalacturonase (PG) activity in alc fruit is reduced to less than 5% of normal, & isozymes PG2a and PG2b are absent in alc fruit. The level of anti-PG precipitable proteins is also reduced to less than 5% of normal. Total polyA+mRNAs is not significantly reduced in ripening alc fruit, but hybridization of polyA+mRNA to each of 15 different ripening-related cDNA clones showed that specific mRNAs are present at 10%-80% of normal levels, depending on the cONA clone used as the probe. PG mRNA was present at 5-10% of the

normal level. All effects of alc on fruit ripening are relieved in the line Alcobaca-red, which arose spontaneously from the original alc line, Alcobaca. The Alcobaca-red trait segregates as a single dominant trait at the alc locus, and is probably the result of a reverse mutation at the alc locus. Each of 18 different ripening-specific clones was used as a probe in an RFLP analysis to determine the number and location of ripening specific loci. 38 such loci were detected, since the number of sites detected per clone ranged from 1-3. The sites were not randomly scattered. There are 5 clusters in which 2-4 ripening loci were located with less than 5% recombination between the outer member of the cluster. However, there is currently no evidence that clusters have any functional basis. The clone for PG was homologous to 1 site on the short arm of chromosome 10. This is moderately linked to the ripening loci alc, nor, and u.

Publications: 88/01 to 88/12

- M. MUTSCHLER, M. GUTTIERI, S. KINZER, D. GRIERSON, and G. TUCKER. 1988. Changes in ripening-related processes in tomato conditioned by the alc mutant. Theor. Appl. Genet. 76:285-292.
- DIBBLE, A., P.J. DAVIES, M.A. MUTSHCLER. 1988. Polyamine content of long-keeping Alcobaca tomato fruit. Pl. Physiology 86:211-216.

12.034 CRISO096935 FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO

TANKSLEY S D; Plant Breeding; Cornell University, Ithaca, **NEW YORK** 14853. Proj. No.: NYC-149313 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 85 to 31 AUG 87

Objectives: Proj. 8500086. Using tomato as a model system, this project will: 1) evaluate the use of monoclonal antibodies as genetic markers to be used in both breeding and genetic experiments, 2) map and characterize additional isozyme markers detected by classical enzyme-activity stains, and 3) incorporate isozyme and monoclonal antibody markers into existing multiple molecular marker stocks.

Approach: Hybridomas will be isolated after inoculation of mice with general, soluble protein from tomato extracts. Monoclonal antibodies derived from individual hybridomas will be labelled with alkaline phosphatase and used to detect their corresponding antigen on Western blots from electrophoresis of crude protein extracts. Mapping will be achieved using existing isozyme and cytogenetic stocks.

Progress: 88/01 to 88/12. A map-based cloning strategy has been devised for isolation and cloning of the Tm-2a gene which confers resistance to tobacco mosaic virus in tomato. Near-isogenic lines have been screened with more than 1000 single copy clones from a PstI genomic library. Seven tightly-linked RFLP clones have now been recovered. Some o "! M ually cover a larger-than-expected portion of the genome. Recombination in this

region of the chromosome is apparently suppressed, possibly because of its proximity to the centromere. CHEF electrophoresis is now being used to link up these markers in the regions of the resistance genes in the anticipation of using this information for eventual cloning of the gene by chromosome walking.

Publications: 88/01 to 88/12

- GANAL, M.W., N.L.V. LAPITAN, and S.D. TANKSLEY. 1988. A molecular and cytogenetic survey of major repeated DNA sequences in tomato (Lycopersicon esculentum). Mole. Gen. Genet. 213:262-268.
- ZAMIR, D. and S.D. TANKSLEY. 1988. Tomato genome is comprised largely of fast-evolving low copy-number sequences. Mole. Gen. Genet. 213:254-261.
- YOUNG, N.D., D. ZAMIR, M.W. GANAL, and S.D. TANKSLEY. 1988. Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the Tm-2a gene in tomato. Genetics 120:579-585.

12.035 CRISO136644 TAGGING PLANT GENES WITH TIGHTLY-LINKED RFLP MARKERS

TANKSLEY S D; YOUNG N D; Plant Breeding & Biometry; Cornell University, Ithaca, **NEW YORK** 14853

Proj. No.: NYC-149309 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 88 to 31 AUG 91

Objectives: PROJ. 8800445. The objective of this research is to find single copy RFLP markers that are tightly linked to several disease resistance genes in tomato. Included in this list of genes are Mi (root-knot nematode resistance), Ve (Verticillium wilt resistance), I/I2 (Fusarium wilt resistance, races 1 and 2) and Tm1/Tm2 (tobacco mosaic virus resistance). Tightly-linked RFLP markers will be oriented into fine structure maps around each resistance gene using pulsed-field electrophoresis. The long-term goal is to develop a map-based cloning strategy which can be used to isolate these and other genes whose gene products are unknown via their position on a saturated RFLP map.

Approach: Tightly-linked RFLP markers will be identified from a single copy genomic library by rapidly testing multiple clones against nearly-isogenic lines. Using this technique, up to 10 clones can be tested simultaneously and we expect to screen 1000-2000 clones during the funding period of this grant. Direct mapping of clones found to be linked to a disease resistance gene will be accomplished using F2 and backcross populations segregating for each gene. Field inversion and CHEF electrophoresis will be employed for physical mapping.

12.036 CRISO095103 FUNCTION OF PLANT RNA-DEPENDENT RNA POLYMERASES

PALUKAITIS P F; Plant Pathology; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-153431 Project Type: HATCH Agency ID: CSRS Period: 28 MAR 85 to 30 SEP 89

Objectives: To determine the relationship of the synthesis of RNA-dependent RNA polymerase to stress. To determine the location and the role of RNA-dependent RNA polymerases. To clone the gene for RNA-dependent RNA polymerase, study its expression and localize its controlling elements.

Approach: Antibodies will be made to purified RNA-dependent RNA polymerase from tomato and will be used: to study the stimulation of the enzyme by various stress inducers; to localize the enzyme; and to screen for an expressed cDNA clone of the enzyme; the cDNA clone will be used both to obtain a genomic DNA clone (from a tomato DNA library) of the RNA-dependent RNA polymerase gene for sequencing purposes, and to study the expression of the gene.

Progress: 88/01 to 88/12. Subviral pathogens such as satellite RNAs and viroids are replicated via RNA polymerases. The recognition sequences involved in the replication process have not been determined. We have cloned three satellite RNAs of cucumber mosaic virus (CMV) that induce different pathogenicities in tomato and replicate to different levels in squash. By recombining the cDNA clones and inoculating tomato plants with infectious RNA transcripts synthesized in vitro from such recombinant DNAs, we have shown that the sequence domains that induce either systemic necrosis or chlorosis are located in different domains of the satellite RNA molecule. In addition, the sequence domain involved in differential replication in squash is located in the 3' half of the satellite RNA molecule, and is distinct from the domains involved in pathogenicity. During replication of the satellite RNAs, tandem, multimeric forms of both (+) and (-) polarity are produced. These multimeric species can be processed to monomeric forms of the satellite RNA, suggesting that these multimers are actually intermediates in the replication of the satellite RNAs. While some satellite RNAs of CMV are replicated faithfully from passage to passage, other satellite RNAs undergo rapid mutation and selection. Both the host species and the strain of helepr virus (CMV) are determinants of the rate and nature of nucleotide sequence variation.

Publications: 88/01 to 88/12

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PALUKAITIS, P. 1988. Pathogenicity regulation by satellite RNAs of cucumber mosaic virus: Minor nucleotide sequence changes alter host responses. Mol. Plant-mic. Interact. 1:175-181.

KURATH, G. and PALUKAITIS, P. 1988. Satellite RNAs of cucumber mosaic virus: Recombinants constructed in vitro reveal independent functional domains for chlorosis and necrosis in tomato. Mol. Plant-Mic. Interact. 1:(in press). PALUKAITIS, P. 1989. Characterization of the replicative intermediates of the satellite RNA of cucumber mosaic virus. Mol. Plant-Mic. Interact. 2:(in press).

12.037* CRISO087911 ALLOZYME AND DNA POLYMORPHISMS AS GENETIC MARKERS IN CROPS

WEEDEN N F; Horticultural Science; N Y Agriculture Expt Station, Geneva, **NEW YORK** 14456.

Proj. No.: NYG632491 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 87 to 30 SEP 92

Objectives: To establish linkage maps using idozyme loci and DNA restriction fragment length polymorphisms in several crop species including garden pea, lentil, common bean, and apple. These biochemically-defined loci will be used to select markers for commercially important traits, identify instances of synteny within the Leguminosae, and to isolate DNA fragments containing other genes of interest.

Approach: Survey the germplasm in beans and apple for new variation, using this variation in crosses to determine the mode of inheritance and linkage relationships and identification of appropriate marker loci. We will pursue the use of cloned DNA fragments to identify restriction=fragment length polymorphisms and use these as additional genetic markers.

Progress: 88/01 to 88/12. Progress continues to be made in the generation of chromosomal linkage maps for garden pea, lentil, apple and grape. In pea, an isozyme locus (ADH-1) was shown to be an excellent marker for En, the locus controlling resistance to pea enation mosaic virus. The location of several previously unmapped isozyme and DNA polymorphisms were determined as well as those of Sn, a gene controlling flowering, and 13 genes involved in nodule formation and nitrogen fixation in pea. A comparison of the preliminary linkage maps for pea and lentil identified several linkage groups in common, suggesting that the parallel mapping of the pea and lentil genomes should work synergistically to increase the rate of progress in either species. Isozyme and DNA markers also have been very useful for genetic studies in apple and grape. In apple, a rudimentary linkage map was developed using isozyme loci and DNA restriction fragment length polymorphisms. We published a genetic analysis of allozyme polymorphisms in grape and demonstrated that techniques initially developed for apple genetics can be applied satisfactorily to similar analyses in grapes.

Publications: 88/01 to 88/12

WEEDEN, N. F. 1988. A suggestion for the nomenclature of isozyme loci. Pisum Newsletter 20:44-45.

WEEDEN, N. F. 1988. Polymorphic isozyme loci identified in Pisum. Pisum Newsletter 20:46-48.

WEEDEN, N. F. and HAGENS, D. 1988. Linkage between Tip-p and Le. Pisum Newsletter 20:42-43. WEEDEN, N. F., KNEEN, B. E., and MURFET, I. C. 1988. Mapping of Sn to chromosome 2. Pisum Newsletter 20:49-51.

WEEDEN, N. F., KNEEN, B. E., and LARUE, T. A. 1988. Mapping genes in Pisum which affect the host plant's ability to form nodules. Genome 30:S287.

12.038 CRISO136033 GERMPLASM ENHANCEMENT AND CULTURE OF EDIBLE MUSHROOMS

ROYSE D J; Plant Pathology; Pennsylvania State University, University Park, **PENNSYLVANIA** 16802.

Proj. No.: PENO3016 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 88 to 30 SEP 92

Objectives: Collect, evaluate, develop, preserve, and make accessible new sources of germplasm; determine genetic distance coefficients and construct genomic maps of linkage groups; develop and improve cultivation technology for specialty mushrooms.

Approach: Lines of edible mushrooms will be collected from word-wide sources by collection and exchange with scientists at other research stations. Emphasis will be placed on reducing production costs of specialty mushrooms through development of improved substrates and optimization of production environments. Trials to determine nutrient preferences for each species will be conducted. Substrate processing will be accomplished using a pasteurizing mixer. Preference will be given to evaluation of selected lines of Agaricus bisporus, Lentinula edodes, Pleurotus spp., and Morchella spp.

Progress: 88/10 to 88/12. Single spore derived 'Pleurotus' spp. isolates from four commercial lines(two 'P'. 'sapidus', one 'P'. 'florida', and one 'P'. 'ostreatus') and from two interspecific hybrids ('P'. 'sajor-caju' x 'P'. 'sapidus') were analyzed for single locus and joint segregation of 25 allozyme encoding loci. The two alleles at the individual loci departed significantly in their segregation from a 1:1 Mendelian ratio in 26% of the intraspecific and 29% of the interspecific tests. Six linkage groups were identified as follows: Dia-1--Est-5; Tpi--Pgd-2--Skdh; (Fum)--Pmg-2--Pgd-1--PepLgg-1--Gr-2; Ndh--Gr-1; Np--PepG1-1--Aat-2--Pgk--Mup; and Gr-4--Mdh-1. The duplicate loci encoding for GR, PEP-LGG, PGM, and PGD were both not linked to each other and not part of duplicate linkage groups. Six loci were not shown to be linked to any other loci (Lap, Pgm-1, Ha, Gpi, PepPap, and PepLgg-2), although the latter two loci were only tested against four and five loci, respecively. The first linkage map of 19 allozyme encoding loci for the 'Pleutorus' genome was developed.

Publications: 88/10 to 88/12
MAY, B., HENLEY, K.J., FISHER, C.G., ROYSE,
 D.J. 1988. Linkage relationships of 19
 allozyme encoding loci within the mushroom
 genus 'Pleutorus'. Genome 30: "in press".

ROYSE, D.J. 1988. Effect of spawn run time and substrate nutrition on yield and size of the shiitake mushroom. Mush. News 36(10):24-30.

12.039 CRISO142023 ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISDZYME AND DNA MARKERS

MUEHLBAUER F J; Agricultural Research Service, Pullman, WASHINGTON 99164.

Proj. No.: 4001-21020-001-73R

Project Type: INHOUSE Agency ID: ARS Period: O7 JAN 87 to O7 JAN 89

Objectives: Assess genetic diversity at isozyme loci and DNA restriction fragments in LENS and CICER. Construct a gene linkage map in LENS and a partial linkage map in CICER. Determine the relative degree to which linkage groups are common to PISUM, LENS and CICER.

Approach: Isozyme loci and DNA restriction fragment length polymorphisms will be used to establish linkage maps in LENS and CICER. Germplasm collections and wildspecies accessions will be screened for isozyme polymorppisms and restriction fragment length polymorphisms. Using the identified polymorphisms, genetic analysis will be performed using F2 populations derived from parents of different genotypes. Linkages for economically important traits such as pod indehiscence and disease resistance will be investigated in order to develop useful markers for breeding purposes.

Progress: 88/01 to 88/12. Nothing additional to report. See report for 'Germplasm, Genetics and Stress Physiology of Food Legumes', CWU 5348-22230-001-00D.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

12.040 CRISO142956 BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS

MUEHLBAUER F J; Agricultural Research Service, Pullman, WASHINGTON 99164.

Proj. No.: 5348-22230-001-02T

Project Type: INHOUSE Agency ID: ARS Period: 01 JUL 88 to 30 JUN 93

Objectives: Develop germplasm & cultivars of grain legumes (peas, lentils, and chick peas) that combine pest resistance, stress resistance and quality. Determine mechanisms of imbibitional stress injury to seeds. Identify generic linkages of isozyme and morphological markers with important genes.

Approach: Use known & newly acquired sources (incl. closely related species) of dis- ease & stress resistance for hybridization & selection to develop multiple disease & stress resistant germplasm & cultivars. The germplasm will be developed in agronomically suitable backgrounds. Physiological & biophy- sical

properties (e.g., imbibition kinetics, deformation & fracture) of legume seed tissues will be measured. Procedure for evaluating imbibitionalstress injury to germinating seeds are being developed. Segregation patterns (especially homologous linkage groups) of morphological genes & isozyme markers will be used to expand the linkage groups, and select for disease resistance genes. Source of funds is WA & ID Dry Pea & Lentil Commission.

Progress: 88/01 to 88/12. PROJECT WAS NEVER FUNDED - WILL TERMINATED ON WANG.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

12.041 CRISO142960 ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND DNA MARKERS

MUEHLBAUER F J; Agricultural Research Service, Pullman, WASHINGTON 99164.

Proj. No.: 5348-22230-001-01T

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Project Type: INHOUSE

Agency ID: ARS Period: O1 APR 88 to 31 MAR 89

Objectives: Assess genetic diversity at isozyme loci and DNA restriction fragments in Lens and Cicer. Construct a gene linkage map in Lens and a partial linkagemap in Cicer. Determine the relative degree to which linkage groups are common to Pisum, Lens and Cicer.

Approach: Isozyme loci and DNA restriction fragment length polymorphisms will be used to establish linkage maps in Lens & Cicer. Germplasm collections and wild species accessions will be screened for isozyme polymorphisms, and restriction fragment length polymorphisms. Using the identified polymorphisms, genetic analysis will be performed using F2 populations derived from parents of different genotypes. Linkages for economically important traits such as pod indehiscence and disease resistance will be investigated in order to develop useful markers for breeding purposes. Trust is with Washington and Idaho Dry Pea and Lentil Commissions.

Progress: 88/04 to 88/12. Nothing additional to report. See report for 'Germplasm, Genetics and Stress Physiology of Food Legumes', CWU 5348-22230-001-00D.

Publications: 88/04 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

12.042 CRISO144289
GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS

MUEHLBAUER F J; Agricultural Research Service, Pullman, WASHINGTON 99164.

Proj. No.: 5348-22230-002-00D

Project Type: INHOUSE Agency ID: ARS Period: 20 JAN 89 to 30 JUL 89

Objectives: Extend existing gene linkage maps of PISUM, LENS and CICER using morphological and molecular markers including restriction

fragment length polymorphisms (RFLPs). Identify molecular marder loci associated with economically important traits.

Approach: Characterize segregating F2 populations from wide crosses for morphological genes, isozyme loci and RFLPs. Use the information to determine linkages and to construct high density gene maps. Use the gene maps to identify "tags" for important genes and to determine regions of the genome that influence quantitative variation. Based on information from these analyses assemble wide crosses expected to enhance and recombine variation for economically important traits. Release enhanced germplasm that possess traits or combinations of traits that are economically important.

12.043 CRISO140267
GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF
FOOD LEGUMES

MUEHLBAUER F J; SPAETH S C; Grass & Legume Genet & Physiolresearch Unit; Agricultural Research Service, Pullman, WASHINGTON 99164. Proj. No.: 5348-22230-001-00D

Project Type: INHOUSE Agency ID: ARS Period: 14 DEC 84 to 14 DEC 89

Objectives: Develop germplasm and cultivars of grain legumes (peas, lentils, and chick-peas) that combine pest resistance, stress resistance and quality. Determine mechanisms of imbibitional stress injury to seeds. Identify genetic linkages of isozyme and morphological markers with important genes.

Approach: Use known and newly acquired sources (including closely related species) of disease and stress resistance for hybridization and selection to develop multiple disease and stress resistant germplasm and cultivars. The germplasm will be developed in agronomically suitable backgrounds. Physiological and biophysical properties (e.g. imbibition kinetics, deformation and fracture) of legume seed tissues will be measured. Procedures for evaluating imbibitional stress injury to germinating seeds are being developed. Segregation patterns (especially homologous linkage groups) of morphological genes and isozyme markers will be used to expand the linkage groups, and select for disease resistance genes.

Progress: 88/01 to 88/12. Allozyme polymorphisms for 18 loci in Lens were investigated and their monogenic inheritance demonstrated. Linkage relationships among these loci and 4 genes controlling morphological traits revealed 6 linkage groups. Several of these groups appear to be conserved among Lens, Pisum and Cicer. A genetic linkage map of lentil comprising 333 cM was constructed using 20 RFLPs, 8 isozyme and 6 morphological markers. Assuming a genome size of 10 morgans, 50% of the lentil genome could be linked to within 10cM of the 34 markers. Allozyme polymorphisms for 13 loci in Cicer were

investigated and monogenic inheritance demonstrated. Linkage analyses showed several small linkage groups. Over 300 germplasm lines of Cicer, mostly C. arietinum, were screened for resistance to Ascochyta blight. About 33 lines exhibited good resistance to the disease. Most recent material originated from USSR. The existence and pathway of cellular pressuredriven extrusion from imbibing seeds was demonstrated using scanning electron microscopy. A method was developed to measure compressive stress which contributes to pressure causing extrusion of intracellular substances. Compressive stress exceeded commonly reported turgor pressure by 2-4 times. An analysis of mechanical stresses in legume seeds during imbibition was begun. A model was developed to describe the coupled uptake by roots of water and solutes and experiments were started to measure parameters needed for the model.

Publications: 88/01 to 88/12

MUEHLBAUER, F.J., N.F. WEEDEN and D.L. HOFFMAN. 1988. Inheritance and linkage relationships of morphological & isozyme loci in lentil (Lens Miller). (Accepted August 1988, Journal of Heredity.).

MUEHLBAUER, F.J., W.J. KAISER and Z. KUTLU. 1988. Collection of Lens and Cicer germplasm in Turkey. (Accepted, 1988, Plant Genetic Resources Newsletter.).

HAVEY, M.J. and F.J. MUEHLBAUER. 1988. Linkages between restriction fragment length, isozyme, and morphological markers in lentil. (Accepted, July 1988, Theoretical and Applied Genetics.).

SUMMERFIELD, R.J., F.J. MUEHLBAUER, and R.W. SHORT. 1988. Controlled environments as an adjunct to field research on lentils (Lens culinaris).

(Accepted by Expl. Agric., 1988.).
HOFFMAN, D.L., F.J. MUEHLBAUER, G.
LADIZINSKY. 1988. Morphological variation
in Lens (Leguminosae). Systematic Botany,
13(1):p. 87-96. Over 3 OO germplasm lines
of Cicer, mostly C. arietinum, were
screened for resistance.

MUEHLBAUER, F.J. 1988. Paper presented at the International Workshop on Breeding Dry Beans Phaseolus vulgaris CIAT, Cali, Columbia, November 7-12, 1988.

SPAETH, S.C. 1988. Extrusion of protoplasm and protein bodies through pores in cell walls of pea, bean and faba bean cotyledons during imbibition. Crop Science, Vol. 29, March-April 1989.

12.044* CRISO090986 STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS

RYAN C A; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO1791 Project Type: HATCH Agency ID: CSRS Period: O1 SEP 85 to 31 AUG 90

Objectives: The objectives of this research are to understand the biochemical and molecular biological basis of insect-induced synthesis and accumulation of proteinase inhibitor

proteins in plants. The complete chemical nature of the wound signals will be sought and their mechanisms of release, transport, intracellular recognition and gene activation will be studied. The structure and organization of the inhibitor genes will be investigated and the wound-induced promoter regions characterized. These promoters will be employed to improve the natural defenses of important crop plants.

Approach: Modern biochemical, immunological and molecular biological techniques will be employed, including recombinant DNA technology.

Progress: 88/01 to 88/12. Two 5' regions of the wound-inducible potato inhibitor II gene that regulate wound-induction have been identified by deletion analysis in collaboration with Dr. Gyn An. These regions, near 650 bp and 150 bp upstream from the translation initiation codon, were shown by gel retardation assays in this laboratory to be the only regions that bind to specific tomato leaf wound-inducible nuclear proteins. One of these trans-factors has been partially purified and has a Mr of 27 kDa. The wound-inducible expression of a fused Inhibitor II-CAT gene in tobacco was shown to be enhanced over 50-fold by sucrose or other metabolizable sugars. This increase is due to transcriptional control, indicating that mRNA synthesis is somehow being regulated by a sucrose-derived molecule. A cDNA coding for wound-inducible trypsin inhibitor in alfalfa leaves (called ATI, a member of the Bowman-Birk inhibitor family) was isolated and characterized. The gene has also been isolated and is currently being characterized. A strongly expressed Inhibitor I gene has been isolated from the DNA of a wild species of tomato, L. peruvianum. This Inhibitor I gene is being introduced into the modern tomato where it is not expressed in fruit. The expression in modern fruit could allow the development of a fruit expression system. Oligosaccharides that induce the accumulation of proteinase inhibitors in plants have been shown to cause the enhanced phosphorylation of plasma membrane proteins from potato and tomato.

Publications: 88/01 to 88/12

CLORE, G.M., GRONEBORN, A.M., NILGES, M. and RYAN, C.A. (1988). The Three-Dimensional Structure of Potato Carboxypeptidase Inhibitor in Solution: A Study Using Nuclear Magnetic Resonance, Distance Geometry and Restrained Molecular Dynamics.

RYAN, C.A. and AN, G. (1988). Molecular Biology of Proteinase Inhibitors in Plants. Plant, Cell and Environment 11:345-349.

RYAN, C.A. (1988). Oligosaccharide Signalling for Proteinase Inhibitors in Plant Leaves. In "Advances in Phytochemistry" (Conn, E., ed.) Vol. 22, Plenum Press, NY, pp. 163-180.

PEARCE, G., LILJEGREN, O. and RYAN, C.A. (1988). Proteinase Inhibitors in Fruit of the Wild Tomato Species L. peruvianum: A possible Mechanism for Plant Protection and Seed Dispersal. Planta 175:527-531.

AN, G., THORNBURG, R.W., JOHNSON, R., HALL, G. and RYAN, C.A. (1988). A Possible Role for 3' Sequences of the Wound-Inducible Potato Proteinase Inhibitor IIK Gene in

Regulating Gene Expression. In "NATO Conference Proceedings".

RYAN, C.A. (1988). Proteinase Inhibitor Genes: Strategies for Manipulation to Improve Natural Plant Defense. BioEssays (in press).

GREENBLATT, H.M., RYAN, C.A. and JAMES, M.N.G. (1988). Structure of the Complex of Streptomyces Griseus Proteinase B andPolypeptide Chymotrypsin inhibitor I from Russet Burbank Potato Tubers at 2.1: A Resolution (in Press).

12.045 THE NATURE OF RESISTANCE TO PLANT VIRUSES

WYATT S D; Plant Pathology; Washington State University, Pullman, WASHINGTON 99164.
Proj. No.: WNPO0495 Project Type: HATCH Agency ID: CSRS Period: O8 NOV 84 to 31 OCT 89

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Objectives: To determine the mechanisms that make PI 186465 cowpeas resistant to cowpea chlorotic mottle virus, CCMV. To determine the molecular basis for the spread of virus in plants and control of virus replication. To determine the genetic difference between natural virus strains and how these differences are expressed.

Approach: To study the resistance of PI 186465 cowpeas to CCMV, the genomes of virulent and avirulent strains are being cloned. cDNAs will be used as probes to study replication and virus movement in the resistant cowpea. The molecular basis for specificity will be determined by comparing nucleotide sequences of virulent and avirulent strains and by the formation of site specific mutants of the virus strains. Antibodies and cDNA probes will be used to study movement of virus and replicational activity within the resistant cowpea.

Progress: 88/01 to 88/12. To study the resistance of PI 186465 cowpeas to cowpea chlorotic mottle virus (CCMV) the P3 gene located on RNA3 is being sequenced. The restriction maps and sequences of strain T (resistant) and R (susceptible) differ quite a bit. Sequence work on several other strains is continuing.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

12.046 CRISO141948 MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONADS

WILLIS D K; Agricultural Research Service, Madison, **WISCONSIN** 53702. Proj. No.: 3655-24000-003-00D

Project Type: INHOUSE Agency ID: ARS Period: 30 MAR 87 to 29 MAR 92

Objectives: Molecular genetic analysis of the genes and gene products involved in pathogenicity, toxin production, and toxin resistance in two phytopatho- genic

pseudomonads, P. s. syringae and P. s. tabaci.

Approach: Phenotypic variants in pathogenicity, tabtoxin production, or tabtoxinine- B-lactam resistance will be generated by transposon mutagenesis. The wild- type genes involved in these processes are isolated from cosmid genomic libraries by either direct restoration of phenotype, or by utilizing the transposon as a molecular hook. The genes will be mapped, sequenced, and the gene products identified. Cloned genes will also be utilized as molecular probes for the investigation of genetic evolution, taxonomic relationships, and disease diagnostic probes. MADISON, WI, PDRR; RM, XX: BL-X; DR. D. K. WILLIS (equipment pending).

Progress: 88/01 to 88/12. The continuing analysis of a mutant strain of Pseudomonas syringae pv. syringae has indicated that syringomycin and/or protease production is involved in lesion formation by this pathogen on bean. This is the first indication of possible biological mechanism for the disease symptoms caused by leaf-spot bacterial pathogens. An recA deficient derivative of a pathogenic P. s. pv. syringae isolate was characterized. This mutation does not significantly alter the pathogenic response. This genetic background is being used in the above analysis of pathogenicity of a recombination deficient background into this system for the study of the molecular genetics of pathogenicity. A DNA region required for tabtoxin production and resistance by both P. s. pv. tabaci (wildfire of tobacco) and P. syringae isolate BR2 (wildfire bean) was recently identified and cloned. This has resulted in the isolation of dianostic DNA probes that distinguish tabtoxin producing strains from nonproducing strains. It has also been established that P. s. angulata can be derived from P.s. pv. tabaci by deletion of the cloned region which suggests that the two major bacterial diseases of tobacco (wildfire and angular leafspot) are caused by derivatives of the same organism.

Publications: 88/01 to 88/12

BARTA, T.M., KINSCHERF, T.G., COLEMAN, R.H. and WILLIS, D.K. 1988. Molecular analysis of a common DNA swquence involved in tabtoxin production by Pseudomonas syringae. Accepted by Phytopathology. Accepted Nov. 3, 1988.

HRABAK, E.M. and WILLIS, D.K. 1988. Effect of a recA mutation...P.s.syringae pv.

syringae on its growth and pathogencity, p. 176. In: N.T. Keen, T. Kosuge, and L.L Walling (eds.) Physio and Biochem of Plant-Micro Inter. Amer. Soc., MD.

WILLIS, D.K., HRABAK, E.M., LINDOW, S.E., and PANOPOULOS, N.J. 1988. Construction and characterization of Pseudomonas syringae recA mutant strains...Molec. Plant-Microbe Interac. 1:80-86.

WILLIS, D.K., KINSCHERF, T.G., BARTA, T.M. and COLEMAN, R.H. 1988. Isolation ...of Tn5 insertions...resistance in P.s. pp. 261-262 In: D.P. Verma, and R. Palacios (ed.). Molecular Plant-Microbe Interactions. APS Press.

- WILLIS, D.K. KINSCHERF, BARTA, T.M. and COLEMAN, R.H. 1988. Molecular analysis of tabtoxin...resistance, p. 163. In: N.T. Keen, T. Kosuge, and L.L. Walling (eds). Physic and Biochem...Amer. Soc. of Plant Path., Rockville, MD.
- WILLIS, D.K., KINSCHERF, T.G., BARTA, T.M. and COLEMAN, R.H. 1988. Molecular genetics of...resistance by Pseudomonas syringae pv. tabaci. J. Cell Biochem. (Abstr). 12C:259.
- HRABAK, E.M., and WILLIS, D.K. 1988. Effect of a recA mutation in P.s. pv.
- syringae on its growth and pathogenicity, p. 176. In: N. Keen, T. Kosuge and L.

12.047 CRUCIFER DISEASES

CRISO027316

WILLIAMS P H; Plant Pathology; University of Wisconsin, Madison, **WISCONSIN** 53706. Proj. No.: WISO0118 Project Type: STATE Agency ID: SAES Period: 01 JUL 85 to 30 JUN 90

Objectives: To develop multiple disease and pest resistant (MDR) lines of cabbage, radish and other crucifers suitable for the fresh and processing markets, fodder and oil. To evaluate the disease, potential and develop control measures for various crucifer diseases.

Approach: Through research on the genetic variability of pathogen populations and studies of the most appropriate conditions of environment pathogen inoculum and host target tissues, develop multiple disease resistance screens that would reliably identify MDR in collections of various crucifer species from around the world. Using appropriate genetic analysis facilitated by our rapid cycling crucifer species stocks, identify and implement the best strategies for incorporating MDR into advanced breeding lines suitable for release to commercial and public plant breeders. To study the etiology and epidemology of various crucifer diseases as they relate to disease control and the development of resistance.

Progress: 88/01 to 88/12. Objectives are met through the following research activities. Maintenance of 14 pathogen species each represented by numerous isolates and pathotypes of crucifer pathogens. Our collection is in excess of 250. Numerous multiple disease resistant male fertile and male sterile of the cole crops cabbage, broccoli and cauliflower, and the Chinese greens, pak choi, petsai, turnip and narinosa have been made available through the Crucifer Genetics Cooperative (CrGC). Fifty advanced breeding lines and cultivars of sauerkraut cabbage were evaluated for 17 varietal traits and resistance to 4 diseases and pests at tree locations in Wisconsin.

Publications: 88/01 to 88/12
BOSLAND, P.W., WILLIAMS, P.H. and MORRISON,
 R.H. 1988. Influence of soil temperature on
 the expression of yellows and wilt of
 crucifers by Fusarium oxysporum. Plant
 Disease 77:777-780.

- BOSLAND, P.W. and WILLIAMS, P.H. 1988.
 Pathogenicity of geographic isolates of
 Fusarium oxysporum from crucifers on a
 differential set of cruicifer seedlings. J.
 of Phytopathology. 123:63-68.
- GLENN, M.G., CHEW, F.S. and WILLIAMS, P.H. 1988. Influence of glucosinolate content of Brassica (cruciferae) roots on growth of vesicular arbuscular mycorrhizal fungi. New Phytol. 110:217-225.
- MONTEIRO, A.A., and WILLIAMS, P.H. 1988. The exploration of genetic resources of Portuguese cabbage and kale for resistance to several Brassica diseases. Euphytica 35:583-592.
- MONTEIRO, A.A., GABELMAN, W.H. and WILLIAMS, P.H. 1988. The use of sodium chloride solution to overcome self-incompatibility in Brassica campestris. Hort. Science 23:876-877.
- SONG, K.M., OSBORN, T.C. and WILLIAMS, P.H. 1988. Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs) I. Genome evolution of diploid and amphidiploid species. Theor. Appl. Genet. 75:784-794.
- SONG, K.M., OSBORN, T.C. and WILLIAMS, P.H. 1988. Preliminary analysis of subspecies within B. rapa (syn. campestris) and B. oleracea. Theor. Appl. Gene.

12.048* CRISO097400 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY

JOHNSON J; ROTH D; BULLA L; Molecular Biology; University of Wyoming, Laramie, WYOMING 82070. Proj. No.: WYO-224-86 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To isolate and to characterize genes in beneficial and pathogenic microbes controlling nitrogen fixation, biological control of pathogens and weeds, host-parasite interactions, host specificity and hypovirulence.

Approach: Although research groups participating in this project are working on different problems and with different organisms, they are all using the same tools of modern molecular genetics and recombinant DNA technology. Before discussing specific procedures which will be used to clone and to characterize genes in plants and associated microbes, it would be useful to briefly discuss the potential and limitations of some of these procedures and techniques.

Progress: 88/01 to 88/12. All projects being carried out have shown substantial progress. Johnson; Frankia N(subscript 2) Fixation-Fragments of Frankia DNA which activate transcription of a LUX cassete have been identified and DNA sequence analyses being done to identify promoter elements. RothJohnson; antisense RNA inhibition of plant virus. Sequences complementary to the 5' region of TMV have been tested in vitro and in vivo for the ability to interfere with TMV gene expression. Roth; TMV replicase activity/protein kinase activities. A unique

protein kinase induced by plant viroids has been identified. Purification and characterization of TMV induced replicase is in progress. Bulla-characterization of B. thuringiensis insecticidal proteins. The genes encoding the insecticidal activity are being cloned into the blue-green algae Anacystis nidulans to develop a self-perpetuating biological insecticide effective against insect vectors of disease.

Publications: 88/01 to 88/12
 CRUM, C.J., JOHNSON, J., NELSON, A. and ROTH,
 D. (1988). Nucl. Acids Res. 16, 4569-4581.
 HIDDINGA, H.J., CRUM, C.J., HU, J., and ROTH,
 D. (1988). Science 241, 451-453.

10

CM 13 ORNAMENTALS AND TURF

13.001 0090545
EXPRESSION OF MELANIN IN PLANTS - MONITORING
GENE EXPRESSION

BOHNERT H J; Biochemistry; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-136334-H-49-081

Project Type: HATCH

Agency ID: CSRS Period: 01 OCT 89 to 30 SEP 92

Objectives: To construct genes that express a protein that leads to a color change in specific plant organs. Plant transformation and monitoring of gene expression.

Approach: Modified genes will be constructed using different promoter regions that drive expression of a polyphenoloxidase coding region. These genes will be stably transformed into ornamental species. Biochemical characterization of the expressed protein and analysis of the products of enzyme activity will be performed.

Progress: 88/01 to 88/12. We are studying the genome and gene organization of the "ancient" plastid found in Cyanophora paradoxa as a model for shuttling genes and gene products between cellular compartments.

Publications: 88/01 to 88/12

BREITENEDER, H., SEISER, C., LOEFFELHARDT, W., MICHALOWSKI, C.B., BOHNERT, H.J. (1988). Physical map and gene map of cyanelle DNA from the second known isolate of Cyanophora paradoxa (Kies strain). Curr. Genetics 13:199-206.

MICHALOWSKI, C.B., RICKERS, J., RAMAGE, R.T., SCHMITT, J.M., BOHNERT, H.J. (1988). Functional replacement in bacteria of a higher plant gene for phosphoenol pyruvate carboxylase. In: Physiol. Suppl. 86:16 (Abstract).

JANSSEN, I., JAKOWITSCH, J., MICHALOWSKI, C., BDHNERT, H.J., LDEFFELHARDT, W. (1988). Sequence Analysis of the Cyanelle PSBA-Gene from Cyanophora paradoxa. The Second International Congress of Plant Molecular Biology, Jerusalem, Nov. 13-18.

13.002

CRISO137050

KELLY J W; BALLARD R E; ABBOTT A G; Horticulture; Clemson University, Clemson, SOUTH CAROLINA 29634.

Proj. No.: SCO1303 Project Type: STATE Agency ID: SAES Period: O1 NOV 88 to 30 JUN 90

Objectives: To isolate and clone RFLP probes that can be as genetic markers in cultivar identification and patent protection.

Approach: RFLPs will be sought by the following methods. One rose cultivar will be chosen to construct a random DNA library using the E. coli plasmid vector pUC8 (Vieira and Messing, 1982). For this purpose, genomic rose DNA will be extracted by the protocol we utilized for all plant and animal taxa we have investigated. The DNA will then be digested and shotgun cloned into pUC8 using the restriction enzyme which gives the best rose DNA digestion

pattern. Individual clones from the DNA library will be characterized by insert size and tested for repetitive sequences. Those that prove to be single copy DNA, i.e., have unique necleotide sequences, will be used to screen for RFLPs by the Southern blotting procedure. It has been our experience that clones carrying 1-2 kb inserts give the highest prevalence of RFLPs with this strategy. To increase the speed of screening varieties, single leaf DNA extracts will be utilized. We have developed a method which gives reasonable DNA yields from 1-2 leaves in peaches.

13.003 CRISO095101 ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS

KENERLEY C M; STACK J P; THOMAS M D; Plant Pathology & Microbiology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6733 Project Type: HATCH Agency ID: CSRS Period: 24 JAN 89 to 31 DEC 93

Objectives: A) Improve the biocontrol attributes of isolates of Gliocladium roseum, G. virens, and G. catenulatum using DNA transformation, protoplast fusion, and other selection procedures. B) Use transformed strains of Gliocladium species that have dominant, stable molecular markers to examine the mechanisms of antagonism. C) Assess the role soil edaphic factors have on propagule formation, survival and dispersal of the target pathogens, and enhanced or engineered antagonists. D) Identify DNA probes that can be used to distinguish the Gliocladium species and strains by RFLP analysis and to physically map selected sites on chromosomes.

Approach: A DNA transformation system developed for Gliocladium spp. will be used to introduce genes (fungicide-resistant, antibiotic production, fungal cell wall degradation) into selected strains of Gliocladium for enhancing biocontrol attributes. Genes will be selected by shotgun cloning by complementation or from other existing fungal systems. Protoplast fusion will be used in cases where genes or metabolic pathways have not been identified (chlamydospore production, sporulation ability, growth potential in soil). Probes for RFLP analysis will be developed from random cloning of genomic digests of Gliocladium spp. as well as using characterized genes cloned from other fungi. The formation of survival propagules and dispersal of fungi mycelium of engineered biocontrol antagonists as well as target hosts will be assessed in soil temperature tanks with a micro-video system.

Progress: 88/01 to 88/12. Protoplast of the biocontrol fungus Gliocladium virens have been transformed with the 6.7 kb plasmid pH1S containing a bacterial hygromycin resistance gene, hygB. Transformants were selected on medium containing 250 mu g/ml hygromycin. Total DAN was isolated from several transformants, digested with restriction endonucleases, blotted to nylon membranes, and hybridized to either purified hygb or pBR322 (vector)DAN. In

most cases the hygB DNA was integrated into high molecular weight DAN and the vector DNA was not present. Also, an episomal, supercoiled, circular plasmid which autonomously replicates has been observed. The plasmid, pJJ31, arose from a spontaneous rearrangement of hygB that had integrated into the genome. It contains new cloning sites for Sal I and Bam HI and can act as a shuttle vector between E. coli and G. virens. Analysis of fusion products of intrastrain crosses of G. roseum revealed that up to six successive hyphal and/or conidial transfers were required to obtain colonies of a homogenous phenotype. All of the tested fusion products were of parental phenotype except for 15 stable His (Asn) and on medium containing benlate and on a medium containing benlate and acriflavin. Of the fusion products tested for their ability to colonize sclerotia of Phymatotrichum omnivorum, 3 performed as well as the control wild-type strain 1620. Several biocontrol agents knows to be antagonistic to Pythium sp. were evaluated for control of damping-off in Amaranthus cruentus by Pythium myriotylum.

Publications: 88/01 to 88/12

- SEH, M.L. and KENERLEY, C.M. 1988. Protoplast formation and regeneration of three Gliocladium species. J. Microbiol, Methods 8:121-130.
- SEALY, R.L., KENERLEY, C.M. and MCWILLIAMS, E.L. 1988. Evaluation of Amaranthus accessions for resistance to damping-off by Pythium myriotylum. Plant Dis. 72:958-989.
- MARTYN, W.R., MORGAN, P.W., STERLING, W.L. and KENERLEY, C.M. 1988. Cotton fleahopper and associated microorganisms as components in the production of stress ethylene by cotton. Plant Physiol. 87:280-285.
- KOCH, D.O., JEGER, M.J., GERIK, T.J. and KENERLEY, C.M. 1987. Spatial dynamics of Phymatotrichum root rot of row crops in the Blackland region of north central Texas. Phytopathology 77:1657-1662.
- JEGER, M.J., KENERLEY, C.M., GERIK, T.J. and KOCH, D.O. 1987. Spatial dynamics of Phymatotrichum root rot in row crops in the Blackland region of north central Texas. Phytopathology 77:1647-1656.

CM 14 CORN

14.001 CRISO131830
ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS
DEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACID

OISHI K K; Plant Science; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-136316-H-25-077

Project Type: HATCH Agency ID: CSRS Period: 01 JUL 87 to 30 SEP 90

Objectives: Maize transposon induced mutations of eight genes (vivipary genes) involved in the regulation or biosynthesis of abscisic acid (plant growth regulator) will be isolated and characterized, the ultimate aim is to use these mutants to clone these genes.

Approach: Using a maize transposon system, Mutator, eight vivipary genes involved in the regulation or biosynthesis of abscisic acid will be mutagenized. 100,000 Mutator maize plants will be self-pollinated and the kernels will be examined for vivipary seeds (lack of seed dormancy due to abscisic acid deficiency). These mutants will be genetically characterized using known vivipary mutants. In addition, to decrease the total number of Mutator elements within the genome, these mutants will be out crossed to inbred lines with zero copies of these elements.

Progress: 88/01 to 88/12. 1. Isolation of Mu tagged maize vp mutants: I have received 13 vp mutants from three laboratories (D. Robertson of Iowa State University, M. Freeling of University of California -Berkeley, and M. Alleman of University of Wisconsin). Eleven of these were in the vp7 complementation group. All eleven were isolated from Mutator plants. The other two mutants in one of two complementation groups, vp1 or vp8. 2. Isolation of vp7 segregating restriction fragments: A restriction fragment length polymorphism has been identified for two alleles of vp7 (vp7*-1 and vp7*-183). From EMBL3 lambda libraries of genomic DNA, several putative clones have been isolated. These are currently being analyzed to determine if these clones contain the vp7 gene.

Publications: 88/01 to 88/12
 No publications reported this period.

14.002 CRISOO96977 EXPRESSION OF MAIZE MITOCHONDRIAL GENOME

WALBOT V; Biological Sciences; Stanford University, Stanford, **CALIFORNIA** 94305. Proj. No.: CALR-8502871 Project Type: CRG0 Agency ID: CRG0 Period: 30 SEP 85 to 29 SEP 87

Objectives: Proj. 8502871. Our objective is to discover new genes in maize mitochondrial DNA.

Approach: Our primary approach to this objective is the production of a lambda gt11 expression library of the entire maize mitochondrial genome. Individual phage are being grown up, and proteins prepared from bacterial cells harboring each phage type. The size of the betagalactosidase protein is determined from each colony, and those colonies

producing a fusion protein are assumed to contain an in frame fusion of a mitochondrial gene with the test bacterial gene. The properties of the mitochondrial DNA encoding such an open reading frame are being studied by determining whether the sequence is transcribed in the plant and by production of antibody against the fusion protein to study the protein product in the plant. Using our approach we can discover genes that are not present in the mitochondrial genomes of mammals or fungi.

Progress: 85/09 to 87/09. The main aims of this project were to define the function of open reading frames in the maize mitochondrial genome and to determine the transcription patterns for several known mitochondrial genes. For the first aim, we have concentrated on the plasmid and episomal molecules of the organelle. We cloned and sequenced the ubiquitous 2.3kb plasmid from the B37 normal cytoplasm. We discovered one large open reading frame on this molecule and two tRNA genes. Further studies are required to determine the function of the open reading frame, but we hypothesize that it encodes a protein bound to the terminal of the plasmid. One of the two tRNAs is for tryptophan and appears by hybridization to be the only such tRNA in the mitochondrial genome; we propose that the 2.3kb plasmid is maintained in all mitochondria because it encodes this required function. We determined the nature of the protein products of the two large open reading frames on the S-2 and S-2 episomes found free in cytoplasmic male sterile S cytoplasms and integrated in the normal cytoplasm. Our primary finding is that the large open reading frames are expressed in both male sterile and fertility restored plants indicating that these products are unlikely to cause male sterility; we also detected the S-2 130kD product in some, but not all, Normal cytoplasms (unpublished data). We have mapped the site of transcription initiation of the co-transcribed 18S+ 5S genes and determined the processing pathway that results in the mature rRNAs.

Publications: 85/09 to 87/09

BEDINGER, P., DE HOSTOS, E.L., LEON, P. and WALBOT, V. 1986. Cloning and characterization of a linear 2.3 kb mitochondrial plasmid of maize. Mol. Ge. Genet. 205:206212.

LEON, P., WALBOT, V. and BEDINGER, P. 198-. Molecular analysis of the linear 2.3kb plasmid of maize mitochondria: apparent capture of tRNA genes. Accepted, Proc. Natl. Acad. Sciences.

MULLIGAN, R.M. and WALBOT, V. 1986. Gene experession and recombination in plant mitochondrial genomes. Tr. Genetics 2:263-266.

MALONEY, A.P. and WALBOT, V. 198-. Structural analysis of mature and dicistronic transcripts from the 18S and 5S ribosomal RNA genes of maize mitochondria. Accepted, J. Mol. Biol.

MULLIGAN, R.M., MALONEY, A.P. and WALBOT, V. 1987. Transcript initiation and RNA processing in maize mitochondria. In press, Mol. Gen. Genetics.

1987. S-2 plasmid of maize mitochondria encodes a 130 kilodalton protein found in both male sterile and fertile plants. In press, Proc. Natl. Acad. Sciences. ZABALA, G. and WALBOT, V. 1987. The S-1 plasmid of maize mitochondria is expressed in male sterile and fertile plants. In press, Mol. Gen. Genetics.

ZABALA, G., O'BRIEN-VEDDER, C. and WALBOT, V.

14,003 CRTS0130001 ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE **ELEMENTS**

WALBOT V; Biological Sciences; Stanford University, Stanford, CALIFORNIA 94305. Proj. No.: CALR-8600172 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 86 to 31 AUG 88

Objectives: PROJECT 8600172. The purpose of this study is to determine what kinds of plant stress and DNA damaging agents can activate cryptic Mutator elements of maize. We have demonstrated that gamma irradiation can effectively reactivate the Mu sequences, and now will explore 5 azacytidine, temperature shock and other treatments. Our hypothesis is that stress-activated transposable element systems may be one way in which plants acclimate and ultimately adapt to a changing environment.

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Approach: We have identified Mutator lines of maize in which this transposable element system has become silenced; this loss of activity is measured as the failure of Mu elements to excise from mutant alleles of genes of the anthocyanin biosynthetic pathway, primarily bronze 1 and 2. When somatic mutability is active, there are many spots of purple pigment on a bronze-colored kernel; when somatic mutability is lost the kernel is uniformly bronze. We discovered that loss of somatic mutability is correlated with an increased level of DNA modification of Mu element DNA. DNA damaging agents and/or stress may result in DNA repair synthesis or even pre-mitotic DNA synthesis not accompanied by the usual modification. Lack of modification may activate the Mu elements again.

Progress: 87/09 to 88/08. During the past year we have finished a major study on the distribution of methylated bases within and flanking the Mu element inserted in the mutable bz2-mul allele. The Bronze-2 gene codes for an enzyme of the anthocyanin biosynthetic pathway. Using transposon tagging, we cloned the bz2-mu1 allele; we have sequenced both the gene and the 1.4 kb Mu insertion (Walbot et al. 1988). Using probes to the Bz2 coding region, we have demonstrated that the Mu element in an active Mutator line is hypomethylated relative to its methylation state in an inactive Mutator background. Alterations in the extent of Mu modification are paralleled by significant changes in the flanking gene sequences, extending for approximately 1 kb from the insertion site. Centrifugation to buoyant density in CsC1 followed by slot blotting of fractions demonstrated that Mu elements in an active line are hypomethylated relative to bulk

DNA while elements in an inactive line acquire the level of modification typical of bulk DNA. We have also demonstrated that mutability in an inactive line can be restored by gamma irradiation; concomitantly, the Mu elements become hypomethylated (Walbot 1988itantly, the Mu elements become hypomethylated (Walbot 1988). We have also shown that in inactive lines Mu elements fail to maintain their copy number (Walbot and Warren 1988).

Publications: 87/09 to 88/08 WALBOT, V., BRITT, A., LUEHRSEN, K., MC LAUGHLIN, M. and WARREN, C.A. 1988. Regulation of mutator activities in maize. In: Plant Transposable Elements (O.E. Nelson, Jr., ed.). Plenum Press, New York. In

press.

WALBOT, V. 1988. Reactivation of cryptic Mutator transposable elements by gamma irradiation. Mol. Gen. Genetics 212:259-264.

WALBOT, V. and WARREN, C. 1988. Regulation of Mu element copy number in maize lines with an active or inactive Mutator transposable element system. Mol. Gen. Genetics 211:27-34.

WALBOT, V., WARREN, C. and BRITT, A. 1988. DNA methylation in maize: Co-ordinate alteration in a Mutator transposable element and flanking gene sequences. In preparation.

14.004 CRISO066763 DEVELOPMENTAL GENETICS USING THE ALCHOL DEHYDROGENASE GENE-SYSTEM IN MAIZE

FREELING M; Genetics; University of California, Berkeley, CALIFORNIA 94720. Proj. No.: CA-B*-GEN-2932-H Project Type: HATCH Agency ID: CSRS Period: O1 NOV 82 to 30 SEP 88

Objectives: Better understand the roles and mechanisms by which genes are used differentially by organ-specific developmental programs. Discover the exact nucleotide sequence or sequence arrangement involved for each regulatory function of a gene. In terms of agriculture: What is the genetic basis for flood resistance? Can particular, transposable sequences from maize be used as integration models for higher plant transformation.

Approach: We use the alchol dehydrogenase-1 (Adhl) gene in maize in two general ways. First, we are interested in finging out the sequences responsible for differential regulation of this household gene. Secondly, we use Adhl as a "trap" for transposable elements. Several maize transponsons have been identified using this approach. Essentially, our approach couples complicated behavior with nucleotide sequences.

Progress: 86/01 to 86/12. This year has been productive. The seven publications are numbered and summarized below: Graduate student Che-Hong Chen published the first paper of his thesis research on the ADH enzymatic and morphological consequences of Ds transposon excisions from exon-4 of ADH. The function of Zn++ ligund is suggested. Not only did we review the general

research area of Mu transposon but presented previously unpublished data on a new Mu transposon, Mu3, and on the relationship between genetic background and Mu methylation. We proved exactly how Mu insertions lower Adhl expression: polymerase II is impeded but chomatin structure is normal. In a collaboration with the Starlinger, Koln lab, we proved that the major anaerobic protein is sucrose synthase A. Our Mu - induced GA3 biosynthetic dysfunctional mutants (dwarves) are described. A high resolution genetic fate map disclosed that a dominant mutant that causes extra epidermal cell divisions actually induces these divisions from cells within the leaf. Mu copy number is under strict controls.

Publications: 86/01 to 86/12

- CHEN, C.-H., FREELING, M. and MERCKELBACH, A. 1986. Enzymatic and morpholigical consequences of Ds excisions from maize. 7aydica XXXI, 93-108.
 LILLIS, M. and FREELING, M. 1986. Mu
- LILLIS, M. and FREELING, M. 1986. Mu transposons in maize. Elsevier Science Publishers B.V., Amsterdam, 183-188.
- VAYDA, M.E. and FREELING, M. 1986. Insertion of the Mul transposable element into the first intron of maize Adhl interferes with transcript elongation but does not disrupt chromatim structure. Plant Molecular Biology. 6:441-454.
- SPRINGER, B., WOLFGANG, W., STARLINGER, D., BENNET, C. and ZOKOLICA, M. 1986. The shrunken gene on chromosome 9 of zea mays L is expressed in various plant tissues and encodes ananaerobic protein. MGG 205:461-468.
- PHINNEY, B.O., FREELING, M., ROBERTSON, D.S., SPRAY, C.R. and SILVERTHORNE, J. 1986.

 Dwarf mutants in maize the gibberellin biosynthetic pathway and its molecular future. Plant Growth Substances: (ed. by M. Bopp).
- HAKE, S. and FREELING, M. 1986. Analysis of genetic mosaics shows that the extra epidermal cell divisions in knotted mutant maize plants are induced by adjacent mesophyll cells. Nature, Vol. 320, No. 6063. 621-623, 17 April 1986.
- ALLEMAN, M. and FREELING, M. 1985. The Mu transposable elements of maize: evidence for transposition and copy number regulation during development.

 Genetics 112: 107-119, January 1986.

14.005 CRISO135932 STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE

HUANG A H C; Botany & Plant Sciences; University of California, Riverside, CALIFORNIA 92521.

Proj. No.: CA-R*-BPS-5006-CG Project Type: CRG0 Agency ID: CRG0 Period: 15 AUG 88 to 31 AUG 91

Objectives: PROJ. 8800331. The project is a study of the structure of genes involved in oil biosynthesis, using maize as a model system. The proposal is designed to provide a basic foundation for future studies of modifying oil quantity and quality.

Approach: Genes of four proteins are targeted. Three of these proteins are the dominant, closely related hydrophobic "structural" proteins of the lipid bodies. The fourth protein is diacylglycerol acyltransferase, which is the only known enzyme unique to triacylglycerol biosynthesis. These proteins will be purified and characterized. Their genes will be studied using selected cDNA and genomic clones. Chromosomal mapping of these genes will also be performed.

14.006 CRISO034385 ISOLATION OF MAIZE NUCLEAR MALE STERILE GENES BY TRANSPOSON-TAGGING

BURGESS D; Advanced Genetic Science Inc.; 6701 San Pablo Ave., Oakland, **CALIFORNIA** 94608. Proj. No.: CALK-8700824 Project Type: SMALL BUSINESS GRANT

Agency ID: SBIR Period: O1 SEP 87 to 29 FEB 88

Objectives: We propose to use transposon-tagging to isolate a nuclear male sterile (ms) gene from maize. We have generated eight new male sterile mutants in stocks carrying the transposable element Activator (Ac) and we are currently analyzing DNA from these mutants for the presence of novel restriction fragments carrying Ac homologous sequences. Preliminary analysis has allowed us to identify several unique restriction fragments which are present in ms mutants but not in sib plants; these new restriction fragments most likely arose as the result of transpositional events.

Approach: During Phase I progency of these mutants will be analyzed to determine whether these restriction fragments co-segregate with male sterility. Once a co-segregating fragment is identified, DNA from that mutant will be size-fractionated and cloned into the appropriate lambda vector. In addition, during the summer months of Phase I a screen for new transposon-induced ms mutants will be set up using translocation stocks in which Ac has been positioned close to the ms locus being targeted.

Progress: 87/09 to 88/11. The goal of this project is to isolate a nuclear male sterile gene from maize using transposon-tagging. For the phase I period four sets of objectives were completed: At the start-up of phase I, eight male sterile mutants had been obtained from on Ac background. In three cases we had already identified Ac-homologous restriction fragments present in the male sterile mutant but not in its sibs. Our first objective was to search for additional Ac-homologous fragments. None were found. Our second objective was to determine whether any of these bands co-segregated with male sterility. Two mutants were analyzed: ms2:6895 and ms2:6893. In both cases the new bands did not co-segregate with male sterility. A third mutant, ms12:6860, could not be analyzed since it did not segregate in a Mendelian fashion. Our third objective was to identify additional male sterile mutants. Seven new male sterile mutants were identified including two in ms14, one in ms7, and one in ms10. Our fourth objective was to examine the new mutants for novel Ac-homologous bands. Both ms14 mutants had transposed Ac's which mapped very close to their site of origin and not to ms14. No new bands were found for either the ms7 or the ms10 mutant.

Publications: 87/09 to 88/11 NO PUBLICATIONS REPORTED THIS PERIOD.

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14.007 CRISOO47923 GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM

CHOUREY P S; NIBLETT C L; Agricultural Research Service; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: 6615-22000-001-01S

Project Type: COOPERATIVE AGREE.

Agency ID: ARS Period: 01 OCT 82 to 30 SEP 87

Objectives: To elucidate gene regulation & expression & to map molecular distributions at the cellular and organismal levels in maize and sorghum.

Approach: Specific parts of the genomes of maize & sorghum will be cloned through re-combinant DNA techniques. These will then be used as probes in DNA:DNA hy- bridization experiments to enable identification of functional roles of specific DNA's.

Progress: 87/01 to 87/09. A region of the maize T cytoplasm mitochondrial DNA (mtDNA) associated with male sterility and disease susceptibility was identified and mapped. The gene atp-6 and co-transcribed genes urf-13-T and ORF25 share promoters by virtue of a 5 Kb repeated region 5' to the genes. Urf13-T was deleted or truncated in all tissue culture-derived mutants to male fertility and disease resistance. The genes atp 6 and ORF 25 were unaffected in the mutants. Antibody to a synthetic peptide, derived from the DNA sequence of urf13-T, immunoprecipitated a 13 kD polypeptide, identifying it as the gene product. The 13 kD polypeptide was absent in all mutants.

Publications: 87/01 to 87/09

TABEIZADEH, Z., PRING, D.R. and VASIL, I.K. 1987. Analysis of mitochondrial DNA from somatic hybrids of Saccarum officinarum (sugarcane) and Pennisetum americanum (pearl millet). Plant Molec. Biol. 8:509-513.

OZIAS-AKINS, P., PRING, D.R. and VASIL, I.K. 1987. Rearrangement in the mitochondrial genome of somatic hybrid cells of Pennisetum americanum (L.) K. Schum. Panicum maximum Jacq. Theor. Appl. Genet. 74:15-20.

SMITH, R.L., CHOWDHURY, M.K.U. and PRING, D.R. 1987. Mitochondrial DNA rearrangements in Pennisetum assoc. with reversion from cytoplasmic male sterility to fertility. Plant Molec. Biol. 9:277-286.

14.008 CRISO034059 CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE

FERL R J; Botany; University of Florida, Gainesville, **FLORIDA** 32611. Proj. No.: FLA-BOT-02402 Project Type: CRGO Agency ID: CRGO Period: O1 AUG 83 to 30 AUG 86

Objectives: Project 8300554. The goal of this project is to gain insight into the mechanisms of genetic regulation. Specifically, this project is designed to probe the changes in chromatin structure that accompany the activation of genes. The alcohol dehydrogenase genes (Adh) of maize will serve as model system.

Approach: The approach is to characterize the chromatin changes that occur when the maize Alcohol dehydrogenase (Adh) genes shift from and inactive to an active state. Nuclease sensitivity is used as an indication of the specific state of the chromatin at the Adh gene location. Changes in chromatin structure will be correlated to changes in gene regulation.

Progress: 87/01 to 87/12. Several methods have been employed to examine the physical structure of the maize alcohol dehydrogenase-1 (Adh1) promoter and its chromatin to asses the possible role(s) of these structures in the regulation of transcriptional activity. Chromatin structure has been examined by in situ digestion of nuclei with restriction enzymes, the promoter DNA has been examined for S1 nuclease sensitivy secondary structures, and the nucleotide sequence has been analyzed by computer modelling. Several correlated structural features have been identified and speculation on their possible regulatory role is presented. DNase-I-hypersensitive sites have been characterized in a plant gene, maize Adhl (which encodes alcohol dehydrogenase 1). It has been generally recognized in animal genes that the chromatin of the 5' flanking region can be characterized by the accessbility of its DNA to the nuclease DNase I (DC 3.1.21.1), indicating which areas in the promoter are "open" to nuclear factors. The 5' region of the maize Adhl gene contains two distinct DNase-I-hypersensitive regions, one constitutively present from position -160 to -700 and one that is anaerobically induced from position -35 to -150. The constitutive region contains three major hypersensitive sites, one of which corresponds in part to a region of potential Z-DNA. The induced hypersensitive region includes TATAA at -38 and CAAT at -100 as well as other potential regulatory sequences.

Publications: 87/01 to 87/12
FERL, R.J. 1985. Architectural
 characteristics of the Maize Adhl Promoter

characteristics of the Maize Adhi Promoter and its Chromatin. Plant Genetics, Pp. 537-546.

PAUL, A.L., VASIL, V., VASIL, I.K. and FERL, R.J. 1986. Constitutive and anaerobically induced DNase-I-hypersensitive sites in the 5' region of the maize Adhl gene. Proc. Natl. Acad. Sci. USA 84:799-803.

14.009 CRISO089829 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT

PRING O R; CHOUREY P S; HIEBERT E; Plant Pathology; University of Florida, Gainesville, FLORIDA 32611.

Proj. No.: FLA-PLP-02317 Project Type: HATCH Agency ID: CSRS Period: 01 DCT 82 to 30 SEP 88

Objectives: Identification and Characterization of Agriculturally Important Genetic Systems. Regulation of Gene Expression and The Delivery of Genetic Material to Higher Plants and Associative Microorganisms. Somatic Cell Genetics and Plant Development: The Modification, Selection Regulation, and Propagation of Plants through Cell and Tissue Culture.

Approach: Plant viral genomes will be mapped and their products characterized. Recognition factors, both host and bacterial, will be characterized. Transposable elements of maize will be used as specific mutagens to identify rate limiting steps in starch biosynthesis. Plasmid-like DNAs in maize and sorghum will be tested as genetic vectors. Plant regeneration from protoplasts and callus will be attempted via organogenesis and somatic embryogenesis.

Progress: 83/10 to 88/09. Promoters and processing sites of maize mitochondrial atp6 are positioned 5' to Turf-13 and ORF221 in T cytoplasm maize, providing regulatory sequences associated with the 13 kD gene product of Turf-13. Maize nuclear backgrounds influence abundance of at least five transcripts associated with the gene. Abundance of the 13 kD gene product is reduced dramatically, while the Rf1 restorer only slightly reduces abundance of major transcripts, suggesting a role of the gene in translation. A maize cell suspension culture was used to study the biology and replication of mitochondrial DNA (mt DNA) and the two minicircular DNAs. All mt DNAs were synthesized rapidly during logarithmic growth phase, whereas no synthesis could be detected in stationary phase. The minicircular DNAs replicated earlier than the principal mt DNA. These data indicate that components of mitochondrial genome exhibit differential replication. Restriction digestion and Southern blot analyses of the bean golden mosaic virus (BGMV) isolated in Florida in comparisons with the Puerto Rican BGMV isolate revealed a high degree of sequence homology between the two isolates. However, distinct restriction patterns with four different endonucleases indicate that the isolates are not identical at the genomic level. A monoclonal antibody prepared to the Florida BGMV was useful in distinguishing some biological variants of BGMV.

Publications: 83/10 to 88/09 KENNELL, J.C., WISE, R.P. and PRING, D.R.

Influence of nuclear background on transcription of a maize mitochondrial region associated with Texas male sterile cytoplasm. Mol. Gen. Genet. 210:399-406. 1987.

- PRING, O.R., GENGENBACH, B.G. and WISE, R.P. Recombination is associated with polymorphism of the mitochondrial genomes of maize and sorghum. Phil. Trans. Royal Soc. London B 319:187-198. 1988.
- SMITH, A.G., CHOUREY, P.S. and PRING, D.R. Replication and amplification of the small mitochondrial DNAs in a cells suspension of Black Mexican Sweet maize. Plant Molec. Biol. 10: 83-90. 1987.
- GILBERTSON, R.L., FARIA, J.C., HIEBERT, E. and MAXWELL, D.P. 1988. Properties and cytology of bean golden mosaic in Brazil. Phytopathology, Abst. 441 submitted for the Annual Meetings of APS, San Diego, CA, Nov. 13-17, 1988.
- CHANG, C.A., HIEBERT, E. and PURCIFULL, D.E. 1988. Characterization and immunological analysis of nuclear inclusions induced by bean yellow mosaic and clover yellow vein potyvirusus. Phytopathology 78, in press.
- CHANG, C.A., HIEBERT, E. and PURCIFULL, D.E. 1988. Analysis of in vitro translation of the bean yellow mosaic virus RNA and the inhibition of proteolytic processing by antiserum to the 49K nuclear inclusion protein. J. gen. Virol.
- HIEBERT, E. and DOUGHERTY, W.G. 1988.
 Organization and expression of the vira.

14.010 CRISO099126 CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE

FERL R J; Vegetable Crops; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-VEC-02582 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 86 to 31 AUG 90

Objectives: PROJ. 8600345. Acquisition of basic knowledge of plant gene structure and function through the in vivo detection of protein/nucleic interactions, DNA architecture and chromatin structure of the maize Adh genes.

Approach: Enzymatic and chemical probes will be used to characterize chromatin. DNA isolated from experiments will be analyzed by gnomic sequencing and Southern blotting.

Progress: 87/10 to 88/09. S1 nuclease was used to probe the architectural characteristics of the maize alcohol dehydrogenase-1 gene promoter. Three sites were identified as hypersensitive to \$1 digestion in supercoiled, but not linear plasmids containing the Adhl gene. The sites mapped to areas located 65, 330 and 800 base pairs 5' to the start of transcription. In each case, the strand specific nicking pattern was determined with nucleotide level precision. An 1830-bp genomic DNA segment containing an Arabidopsis thaliana actin gene AAcl, has been cloned and sequenced. The AAcl gene is present as a single-copy gene, but at least two other actin-like genes have been detected. Comparison of the nucleotide sequence of AAcl with other cloned plant actin genes reveals four exons separated by three introns conservatively located in all plant actin genes. The deduced amino acid sequence has also been compared with actin protein sequences from other plants.

Publications: 87/10 to 88/09

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NAIRN, C.J., WINESETT, L., and FERL, R.J. 1988. Nucleotide sequence of an actin gene from Arabidopsis thaliana. Gene, 65:247-257.

FERL, R.J., NICK, H.S. and LAUGHNER, B.H. 1987. Architecture of a plant promoter: Sl nuclease hypersensitive features of maize Adhl. Plant Mol Biol 8:299-307.

14.011 CRISO099210 MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE

HANNAH L C; Vegetable Crops; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-VEC-02588 Project Type: CRGO Agency ID: CRGO Period: 01 AUG 86 to 31 JUL 89

Objectives: PROJ 8600449. Determine the sequence of the exons, introns and promoter of the constitutive sucrose synthase gene (Css) of maize, examine the differential expression of two sucrose synthase genes in various parts of the maize plant and map Css to one to the maize chromosomes.

Approach: A molecular clone containing genomic sequences of the Css locus has been isolated. This will be used to isolate a molecular clone for the mRNA of Css. Both clones will then be sequenced. Fragments at the 5' end of the gene will be used in primer extension studies to identify the promoter of Css. Clones above and clones for the Sh1 gene will be used measure mRNA levels in various plant parts. A restriction length polymorphism, identified by us previously will be genetically mapped in order to place this locus on the genetic map of maize.

Progress: 87/10 to 88/09. The Sus locus encodes a sucrose synthase found in many parts of a maize plant. Cloning and sequencing of genomic and cDNA clones for Sus revealed the following information. There exists very strong sequence similarity between exons of Sus and Sh1. Little sequence homology is found in introns. Sizes of the corresponding introns are approximately the same. Intron 15 of Sh has no counterpart in Sus. Examination of Sh shows much sequence similarity between intron 15 and exon 16 of Sh. This suggests that a duplication occurred in Sh, which did not occur in Sus. The cloning of the Sus gene from the maize inbred W22 yielded a Sus hybridizing clone which is fundamentally different from Sus. The size and pattern of hybridization between this and an authentic clone of Sus, suggests that this may be a pseudo gene of Sus which lacks introns. Developmental profiles of Sus through maize development shows that transcript levels, as a fraction of total RNA, peek early in endosperm development and do not rise during the period of active starch synthesis. Furthermore, a very large transcript with sequence similarity to Sus if found at later stages of endosperm development.

Publications: 87/10 to 88/09

HAUPTMANN, A.M., VASIL, V., HANNAH, L.C., VASIL, I.K., FERL, R. 1988. Promoter strength comparisons of maize shrunken 1 and alcohol dehydrogenase 1 and 2 promoters in mono-and dicotyledonous species. Plant Physiol. (in press).

KLEIN, A.S., CLANCY, M., FURTEK, D.B..
HANNAH, L.C. and NELSON, O.E. 1988. The
mutation bronze-mutable 4 Derivative 6856
in maize is caused by the insertion of a
novel 6.7 kilobase pair transposon in the
untranslated leader region.

HANNAH, L.C., MCCARTY, O.R. 1988. Mature pollen contains transcript of the consitutive sucrose synthase (Css) gene. Maize Genetics Cooperation Newsletter 62:59

BOURNIVAL, B.L., VALLEJOS, C.E., CHOUREY, P.S., HANNAH, L.C. 1988. An activity stain for sucrose synthase. Maize Genetics Cooperation Newsletter 62:60.

COBB, B.G., HANNAH, L.C. 1988. Shrunken-1 encoded sucrose synthase is not required for sucrose synthesis in the maize endosperm. Plant Physiology (in press).

BAIER, J.W. 1988. Thesis. University of Florida. DNA sequence differences in wild type alleles of the shrunken gene of maize.

14.012 CRISO136023 DISSOCIATION MUTAGENESIS OF THE SHRUNKEN-2 LOCUS OF MAIZE

HANNAH L C; Vegetable Crops; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-VEC-02772 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 88 to 31 AUG 90

Objectives: PROJ. 8800524. Clone and characterize the shrunken-2 (Sh2) gene of maize. Clone the brittle-2 (Bt2) gene of maize. Create variability in the shrunken-2 gene by passage of the transposable element Ds through the locus.

Approach: Full length cDNA clones and genomic clones of Sh2 will be isolated from a cDNA library in lambda GT-10 and from a genomic lambda library of the genotype Sh2-Prog. Sequencing will be done in M13 using the dideoxy method. Cloning of Bt2 will be via homology to a rice ADP-glucose pyrophosphorylase clone. Mutagenesis will be done in field grown corn using Ac-Ds stocks in which Ds originally resided at the A1 and Sh2 loci.

14.013 CRISO034161 CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST

STILES J I; Botany; University of Hawaii, Honolulu, HAWAII 96822.

Proj. No.: HAW00652-G Project Type: CRG0 Agency IO: CRG0 Period: O1 AUG 83 to 31 OCT 85 **Objectives**: PROJ 8200534-1. To construct a cloning vector capable of expressing cloned sequences in yeast. To use this vector to isolate maize genes coding for amino acid and nucleic acid biosynthetic enzymes. To characterize these maize genes using genetic and recombinant DNA procedures.

Approach: A cloning vector capable of selection and replication in either E. coli or S. cerevisiae will be constructed. This vector will have a cloning site adjacent to a yeast promoter such that double stranded cDNA clones will have a high probability of transcription. The vector has also been arranged such that translational signals should be present. Specific maize genes will be selected by biological complementation of markers in the host yeast strain. The maize sequences will then be analyzed using genetics and recombinant DNA and related nucleic acid techniques such as DNA sequencing and transcription mapping.

Progress: 85/01 to 85/09. A vector capable of expressing foreign genes in yeast has been constructed and tested. This vector has sequences allowing growth and selection in both E. coli and yeast. It also contains a modified yeast iso-1-cytochrome c gene in which the coding region has been deleted and replaced with a synthetic coding EcoR1 linker. Sequences inserted at this site will be transcribed from the iso-1-cytochrome c promoter with proper termination of transcription. A gene coding for the wheat storage protein alpha-gliadin has been inserted into the vector to assess its function. Yeast containing this vector synthesize abundant quantities of alpha-gliadin mRNA. The size of the message is as predicted if transcription begins at the expected position in the iso-1-cytochrome c promoter, proceeds through the alpha-gliadin sequences and terminates at the normal position in the iso-1-cytochrome c 3' region. The transcripts also show repression if the yeast is grown on high glucose as would be expected if the iso-1-cytochrome c regulatory region is directing transcription. Immunological techniques have shown that about 0.1% of the total yeast protein in transformed strains is alpha-gliadin and that the size of the yeast-produced alpha-gliadin is identical to that isolated from wheat flour. Since alpha-gliadin contains a single peptide, it is recognized and cleaved in yeast at approximately the same point as in wheat.

Publications: 85/01 to 85/09
NO PUBLICATIONS REPORTED THIS PERIOD.

14.014 CRISO140392 BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIDS, AND NATURAL ANTIOXIDANTS IN CORN

WILSON C M; WEBER E W; Agricultural Research Service; Agricultural Research Service, Urbana, ILLINOIS 61801.

Proj. No.: 3611-23000-001-00D

Project Type: INHOUSE

Agency ID: ARS Period: 15 NOV 84 to 30 SEP 87

Objectives: Develop assays for protein and oil in corn; develop knowledge of quantity and quality of protein and oil in corn seed; investigate metabolism and inheritance of proteins, lipids, and natural antioxidants in corn; develop basic knowledge needed to increase the quality of the corn crop.

Approach: Identify zein polypeptides by isoelectric focusing banding pattern and apparent molecular mass, develop a classification system for the zeins, andrelate the different zeins to genotype, high-lysine mutant protein regula- tors, and chromosomal gene locations. Determine the variability and inher-itance of tocols in corn grain and the interrelationships between levels ofpolyunsaturated fatty acids, oil stabilities, germinabilities, and individ-ual and total tocols. Determine the variability of individual and total carotenoids among various corn strains and the relationship of the carotenoids to oil content, protein content, and proportion of vitreous endo- sperm in the kernel.

Progress: 87/01 to 87/09. 70 zeins were identified by isoelectric focusing (IEF) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A genetic linkage map constructed by multi-dimensional scaling showed 11 to 13 zeins linked on chromosomes 4 and 3 to 5 linked on chromosome 7. IEF and SDS-PAGE analyses of several commercial zeins revealed that an appreciable fraction had not suffered degradation during the isolation of the corn gluten meal and subsequent extraction of zein. These studies on commercial zeins will aid in the development of uniform products which may lead to increased commercial utilization of zein in various food uses. Two minor low-molecular weight zeins which differ from previously identified zeins of similar sizes were detected using IEF and SDA-PAGE. A recommended zein nomenclature was submitted to an international committee for consideration. The concentrations per dry wt of total tocols and carotenoids in developing corn grain were highest from 30-40 da after pollination. The loss of carotenoids in developing corn grain were highest from 30-40 da after pollination. Loss of carotenoids after storage at room temp for 6 mo varied among 4 inbred lines from 33-50%. Individual tocols and carotenoids were found to vary widely among corn inbreds. Corn lines have been identified that have high levels (>90%) of alpha- or gamma-tocopherols. High alpha-lines have lowered levels of total tocopherols.

14.015 CRISO099729
CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT
LENGTH POLYMORPHISMS (RFLPS)

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WEBER D S; HELENTJARIS T G; School of Biological Sciences; Illinois State University, Normal, **ILLINOIS** 61761.

Proj. No.: ILLR-8601845 Project Type: CRGD Agency ID: CRGD Period: 15 SEP 86 to 14 SEP 89

Objectives: PRDJECT 8601845. This project will further earlier collaborative efforts between our two research groups (a cytogenetics group and a molecular biology group) in developing a genetic map for maize based upon restriction fragment length polymorphisms (RFLPs).

Approach: Using primarily randomly-isolated unique sequence clones as hybridization probes, informative or "polymorphic" loci will be detected and arranged into genetic linkage groups. These will then be assigned to the chromosomes which contain them by analysis of their presence in monosomic individuals. Drientation and internal correlation with the conventionally-derived linkage map will be accomplished through analysis of these loci with B-A translocations and other chromosomal rearrangements and with known morphological marker stocks. The resulting clones can also be used for many other future applications within our own groups as well as through their distribution to other investigators.

Progress: 87/01 to 87/12. Monosomic maize plants were generated using the r-X1 deficiency, and DNAs extracted from monosomics and the parents of the monosomics were used in restriction fragment length polymorphism (RFLP) studies. Randomly-isolated unique sequence clones and cDNA clones were assigned to the appropriate linkage groups using monosomics. Linkage relationships between clones located on the same chromosome were determined by linkage analysis. Sixty-two of the 217 cloned sequences analyzed hybridized at more than one location in the maize genome; thus, more than one nucleotide sequence is present within the maize genome which is in part homolgous to each of these cloned sequences. We determined the genomic locations of each of these "duplicate" sequences and found that they usually were located on different chromosomes. The process which produced them did not operate randomly as some pairs of chromosomes share many duplicate sequences while many other pairs share none. Furthermore, certain chromosomal segments share several duplicate sequences in an ordered arrangement suggesting that these segments may have had a common origin. However, the duplicate RFLP loci do not primarily involve five pairs of chromosomes; thus, the maize genome currently does not contain five pairs of homoloeous chromosomes. We have also utilized B-A translocations to determine the long and short arms of each of our linkage groups and to correlate our RFLP map to a certain extent with the conventional map.

Publications: 87/01 to 87/12
HELENTJARIS, T., WEBER, D. and WRIGHT, S.
1988. Identification of the genomic
locations of duplicate nucleotide sequences
in maize by analysis of restriction

fragment length polymorphisms. Genetics, in press.

WEBER, D., HELENTJARIS, T. and WRIGHT, S. 1987. Duplicate sequences in maize identified and mapped through the use of restriction fragment length polymorphisms (RFLPs). Genetics 116:s10 (abstract).

14.016 CRISO141589
MAIZE GRAIN PROTEIN COMPOSITION AND
DISTRIBUTION AS RELATED TO HARDNESS, HANDLING
AND PROCESSING

BIETZ J A; PAULIS J W; WU Y V; Northern Regional Res Center, Peoria, ILLINOIS 61604. Proj. No.: 3620-41000-010-00D

Project Type: INHDUSE Agency ID: ARS Period: O1 APR 86 to 30 JUN 89

Objectives: To establish the relationship of corn endosperm protein composition and distribution to resistance of grain to breakage during handling, and to yield of desired kernel components during industrial processing. To develop standards for breeders to attain in improving corn quality.

Approach: Relate hardness and breakage susceptibility of normal and mutant corn genotypes with flint, dent and floury endosperms to protein distribution and deposition. Analyze proteins of lines varying in hardness and grits yield by electrophoresis and HPLC; develop practical rapid methods to identify desirable genotypes. Examine glutelin disulfide crosslinking. Relate endosperm morphology during maturation to genotype and control of protein synthesis and deposition. Relate proteins to quality in experi- mental flint or vitreous lines. Explore regulation of protein synthesis and deposition in normal and opaque-2 corn at molecular level. Mill and air-classify grain and relate particle size to hardness.

Progress: 88/01 to 88/12. We analyzed maize F1 crosses and F2 meals by RP-HPLC to measure inheritance of alcohol-soluble proteins. Computer derived chromatograms of hybrids closely resembled those of authentic samples. In crosses with modifier genes, however, some polypeptides did not show a linear dose-response relationship, suggesting complex regulation of protein synthesis. HPLC of sorghum proteins revealed a protein which may relate to hardness. We are also identifying maize proteins related to hardness. Maize lines were milled and air-classified to relate particle size distributions to hardness. Reverse osmosis and ultrafiltration of corn stillage solubles economically produced protein-rich concentrates. HPLC + IEF separated zein by hydrophobicity and charge, revealing more heterogeneity than had been previously obseved. Sugary-1 maize lines all contained zein A1/30.5 when homozygous for su-1. Illinois Low Protein maize and seeds produced on low nitrogen were low in zein, but relatively rich in high-methionine proteins. In a cooperative study, zeins in recombinant inbreds were mapped to chromosomes by RFLP analysis. Open-pollinated Reid Yellow Dent and Lancaster

lines contained 23-25 zeins. Additional crosses were made to map additional zeins. Hard and soft endosperm fractions were isolated from a hybrid of known breakage susceptibility, and albumins, globulins, prolamins and glutelins were extracted. These proteins are being related to endosperm vitreosity.

Publications: 88/01 to 88/12
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WILSON, C.M., SPRAGUE, G.F. and NELSEN, T.C. Linkages among zein genes determined by isoelectric focusing. Theor. Appl. Genetics, Accepted 10-17-88.

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WALL, J.S., COOKER, L.A. and BIETZ, J.A. 1988. Structure and origin of maize endosperm alcohol-insoluble glutelin. J. Agric. Food Chem. 36:722-728.

WALL, J.S. and CARPENTER, K.J. 1988.

Variation in availability of niacin in grain products. Food Technol. 42:198-204.

PAULIS, J.W., BIETZ, J.A., BOGYO, T.P., DARRAH, L.L. and ZUBER, M.S. 1988.
Expressivity of endosperm protein genes in single and double mutant maize endosperm.
Genome 30, (1) (Abstract 35.31.27).

BOGYO, T.P., PAULIS, J.W., BIETZ, J.A., DARRAH, L.L. and ZUBER, M.S. 1988. Expressivity of zein genes in single-and double-mutant maize endosperm. Genome 30 (1) (Abstract 35.31.26).

14.017 CRISO095743 ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS

HEPBURN A G; Agronomy; 1301 West Gregory
Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-15-0363 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To isolate agronomically important genes of known function from crop species or from species related to crops species. To insert known autotransposing elements into plant species showing agronomically significant characters and to screen for transposon mutagenesis of those characters.

Approach: Bacteriophage Lambda expressing libraries will be made of cDNA from plant species and screened using antibodies raised against important proteins. To induce transposon mutagenesis, a known plant transposable element (Mu, Ac or Tam 3) will be inserted into the host species genome using disabled Agrobacterium vectors and the resultant tissue or regenerated plants screened for evidence of transposon inactivation of specific characters.

Progress: 87/10 to 88/09. The induction of genes in soybean plants cultured cells in response to culture filtrate of the pathogenic

fungus Phialophora gregata has been studied. cDNA libraries have been produced and ten clones selected that are induced in resistant plants following toxin exposure. These clones isolated originally in bacteriophage lambda gt10 have been recloned into a pasmid vector. The clones have been used as probes against mRNA preparations isolated from resistant and sensitive cultivars of soybean innoculated with the fungus. The alterations in the expression of the genes corresponding to the clones has been studied with a view to identifying genes that may correspond to the primary resistance response. A clone of the soybean chalcone synthase gene was used as a control. Clone G10-1 was the only clone which showed specific induction in the leaves of resistant plants treated with the fungus. Clone G5-2 was induced in stems of either susceptible plants (Corsoy) inoculated with a nonpathogenic fungal isolate (It) or resistant plants (PI437833) inoculated with a pathogenic isolate (C4). Each of these represents a nonproductive infection and hence this clone may be involved in the response to fungal attack. As part of our studies to isolate and identify important genes in crop species, we have also been examining the DNA transfer capability of Agrobacterium tumefaciens with a view to developing more efficient transformation vectors. It is hoped that such vectors will help to overcome the low DNA transfer efficiency from A.

Publications: 87/10 to 88/09 MONTE-NESHICH, D. C. Molecular reponse of soybean (Glycine max (L.)) to Phialophora gregata. Ph.D. Thesis, University of Illinois at Urbana-Champaign, (1988). CULIANEZ-MACIA, F. C. and HEPBURN, A. G. (1988) Right-border sequences enable the left border of an Agrobacterium tumefaciens nopaline Ti-plasmid to produce single-stranded DNA. Plant Molecular Biology 11:389-399. CULIANEZ-MACIA, F. C. and HEPBURN, A. G. (1988) The kinetics of T-strand production in a nopaline-type helper strain of Agrobacterium tumefaciens, Molecular Plant-Miscrobe Interactions 1:207-214.

14.018 CRISO0121111 STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE & THEIR USE AS RESEARCH TOOLS

PATTERSON E B; Agronomy; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801. Proj. No.: ILLU-15-0388 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 82 to 30 SEP 87

Objectives: Map, study transmission characteristics and preserve useful derived stocks of selected reciprocal translocations. Isolate, map and study transmission of duplicate-deficient chromosome complements. Locate and map select unplaced genes.

Approach: Emphasis will be placed on translocations that will generate egg-transmissible duplicate-deficient (Dp-Df) chromosome complements. Translocations will be mapped relative to marker genes. Information on transmission of aneuploid chromosome

complements will be obtained. Linkage and transmission characteristics of T9-10a will be summarized as a type example of the behavior and uses of translocations that transmit Dp-Df chromosome complements. A chromosomally-modified version of Dp-Df 9-10a will be evaluated for use in avoiding detasseling in production of hybrid corn. Translocations with an interchange point near the tip of a chromosome arm will be screened to identify those yielding egg-transmitted deficiencies. Select unplaced genes will be located to chromosome using chromosome rearrangements.

Progress: 82/01 to 87/09. The following genes have been located to chromosome: Ht, Rp3, Rp4, Rp5 (genes for disease resistance); f12 (balanced protein); ms5, ms7, ms9, ms10, ms11, ms12, ms13, ms14 (male steriles). -- About 80 female-transmissible duplicate-deficient (DpDf) chromosome complements generated from reciprocal translocations or inversions have been identified. About 50 of these uncover the gene po in 6S, yg2 in 9S or sr2 in1OL. -- DpDf plants are heterozygous deficient for the chromosome segment distal to one of the interchange points and are triplicated for the segment distal to the second interchange point. Procedures have been described whereby the positions of genes in either direction from the two interchange points may be determined without classifying testcross progeny for the presence of the interchange -- About 250 reciprocal translocations were tested for generation of DpDf complements linked to one of 10 selected male sterile gene loci. About 40 linkages were detected; four that gave recombination values of about two per thousand or less provided stocks for implementing a subsequently-patented DpDf-ms linkage technique for producing male sterile seed stocks in normal cytoplasm for production of commercial hybrids without detasseling.

PATTERSON, E.B. 1968. Location of f12. Maize Genet. Coop. Newsl. 42:42-44.

PATTERSON, E.B., HOOKER, A.L., SAXENA, K.M.S. and YATES, D.E. 1968. Linkage relations of Ht; mapping studies of Rp3, Rp4 and Rp5. Maize Genet. Coop. Newsl. 42:44-51.

PHILLIPS, R.L., BURNHAM, C.R. and PATTERSON, E.B. 1971. Advantages of interchanges that generate haplo-viable deficiency-duplications. Crop Sci.

Publications: 82/01 to 87/09

1:525-528.

PATTERSON, E.B. 1978. Properties and uses of duplicate-deficient chromosome complements in maize. In: Maize Breeding and Genetics, D.B. Walden (ed.). John Wiley, New York.

PATTERSON, E.B. 1982. The mapping of genes by the use of chromosome aberrations and multiple marker stocks. In: Maize for Biological Research, pp.

85-88, William F. Sheridan, ed., University of North Dakota Press, Grand Forks.

14.019 CRISO135356 ANALYSIS OF GENE EXPRESSION IN DEVELOPING MAIZE EMBRYOS

KRIZ A L; Agronomy; 809 South Wright Street, Champaign, **ILLINOIS** 61820.

Proj. No.: ILLU-15-0514 Project Type: CRGO Agency ID: CRGO Period: 01 JUN 88 to 31 MAY 91

Objectives: PROJ. 8800629. The primary objective of this work is to gain an understanding of gene regulation during embryo development in maize (Zea mays L.).

Approach: This work involves characterization of genes encoding embryo-specific globulins. These proteins accumulate to high levels during seed development so that they are the major proteins in mature embryos. Standard recombinant DNA procedures will be used to isolate and characterize DNA clones corresponding to globulin-specific genes. By examining the expression of these genes in normal and mutant maize embryos, we hope to develop an understanding of factors involved in specific gene regulation during seed development. Analysis of these genes at the nucleotide sequence level may allow for identification of certain regions of these genes that are important for embryo-specific gene expression. These studies will eventually be extended to investigate molecular processes involved in maize embryogenesis.

Progress: 88/06 to 88/09. This project was started on June 1, 1988. Prior to that date, we had constructed a cDNA library specific for mRNA from developing maize embryos and identified clones corresponding to the Glb1 (formerly Prot) locus, which encodes the major globulin storage protein of the embryo. During the period covered by this report we determined the entire nucleotide sequence of a partial cDNA clone corresponding to Glb1. Comparison of amino acid sequence, as deduced from the nucleotide sequence, with direct protein sequence data confirmed that this clone corresponds to Glb1. Using this clone as a probe in Northern blot analysis of maize embryo RNA, we found that the Glb1 transcript is present at high levels throughout most of embryo development. Similar analysis of RNA from embryos homozygous for a Glb1 null allele, which contain no detectable Glb1-encoded protein, revealed that these embryos produce a low amount of Glb1-specific transcript which is a different size than that produced by embryos possessing functional alleles of the gene. This suggests that the defect in the null allele is at the transcriptional and/or post-transcriptional level of gene regulation. Characterization of the null allele at the nucleotide sequence level may provide significant insight with respect to gene regulation in higher plants. For such characterization, we have obtained genomic clones corresponding to two functional Glb1 alleles, as well as the null allele, and these are currently being subjected to nucleotide sequence analysis.

Publications: 88/06 to 88/09

No publications reported this period.

14.020 CRISO073243 REGULATION OF GENE EXPRESSION IN PLANTS

LARKINS B A; Botany & Plant Pathology; Purdue University, West Lafayette, **INDIANA** 47907. Proj. No.: INDO55044 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 82 to 30 SEP 88

Objectives: Determine the relationship between gene structure and the transcriptional regulation of gene expression in important crop plants. Analyze the relationship between gene organization and the developmental regulation of gene expression. Determine the structural relationships between storage protein genes in major crop plants and their evolutionary relationships. Analyze the nature and contribution of trnascriptional control mechanisms in protein synthesis as they relate to seed protein accumulation.

Approach: Materials for study will be obtained from the field and greenhouse for analysis in the laboratory. Recombinant clones of plant DNA will be constructed. Clones corresponding to seed protein and other genes will be isolated and characterized with respect to structure and function. These clones will be used to study the regulation of gene activity during seed development.

Progress: 87/10 to 88/09. The storage proteins of maize seed, zeins, can be divided into four types that we distinguish as alpha, beta, gamma, and delta, on the basis of primary amino acid sequences. The alpha zeins are encoded by a large multigene family, while the others are encoded by only one or two genes. Zeins account for half the total endosperm protein, and because they contain no lysine maize is of poor nutritional quality. Several mutations have been identified that cause a reduction the synthesis of alpha zeins and as a consequence result in a higher proportion of lysine in the kernel. Part of our research effort has been to investigate the mechanisms by which these mutations affect the expression of zein genes. We have found that one of these mutations, opaque-2, causes a block in the transcription of alpha zein genes. Interestingly, it does not affect the expression of the gamma zeins. We have also studied an altered phenotype of opaque-2 called QPM, or "quality protein maize". These modified opaque-2 mutants have a normal phenotype yet maintain a high lysine content. Part of the explanation for the normal phenotype is that there is a doubling of the expression of the gene encoding the gamma zein. This indicates that, while the product of the opaque-2 locus is probably a transcriptional regulatory factor, it does not affect expression of all zein genes. We have also found that an unusual pattern of zein synthesis occurs within the endosperms of the modified opaque-2 mutants relative to their normal counterparts.

Publications: 87/10 to 88/09

GALILI, G., KAWATA, E.E., SMITH, L.D., and LARKINS, B.A. Role of the 3' poly(A) sequence in transcriptional regulation of mRNAs in Xenopus laevis oocytes. J. Biol. Chem. (1988) 263, 5764-5770. LENDING, C.R., KRIZ, A.L., LARKINS, B.A., and BRACKER, C.E. Structure of maize protein bodies and impupocytochomical legalization.

bodies and immunocytochemical localization of zeins. Protoplasma (1988) 143, 51-62. WALLACE, J.C., GALILI, G., KAWATA, E.E., CUELLAR, R.E., SHOTWELL, M.A., and LARKINS,

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UENG, P.E., GALILI, G., SAPANARA, V., GOLDSBROUGH, P.B., DUBE, P., BEACHY, R.N., and LARKINS, B.A. Expression of a maize storage protein gene in petunia plants is not restricted to seeds. Plant Physiol. (1988) 86, 1281-1285.

WALLACE, J.C., GALILI, G., KAWATA, E.E., LENDING, C.R., KRIZ, A.L., BRACKER, C.E., and LARKINS, B.A. Location and interaction of the different types of zeins in protein bodies. Bichem. Physiol. Pflanzen (1988) 183, 107-115.

14.021 CRISO131030 MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS

LEE M; LAMKEY K R; POLLAK L; Agronomy; Iowa State University, Ames, **IOWA** 50011. Proj. No.: IOW02818 Project Type: HATCH Agency ID: CSRS Period: 01 FEB 87 to 30 SEP 91

Objectives: To obtain information on: allelic constitution of public maize inbred lines for genomic DNA probes used to develop the RFLP linkage map, collating the linkage map for RFLPs and isozymes, the relationship between RFLPs, combining ability, and heterotic pattern of inbred lines and populations, the degree of relationship and genetic diversity among inbred lines using RFLPs, linkages between molecular markers and important single, major genes, changes in allele frequency in populations developed through long-term recurrent selection programs, relationships between changes in allele frequency and changes in traits under selection.

Approach: Survey the genomic DNAs of widely used public maize inbreds for several probe-restriction endonuclease combinations using Southern hybridizations with radiolabeled (p32) genomic probes against genomic digests of the inbreds, create F(2) populations using inbred parents with known RFLP and isozyme polymorphisms, produce F(1) hybrids, population crosses using germplasm with established molecular polymorphisms and measure their performance in agronomic trials, and cluster analysis of inbred lines and populations based on allelic constitution at the molecular level.

Progress: 88/01 to 88/12. A set of 27 elite dent corn inbreds were subjected to RFLP analysis at 47 loci. All loci were polymorphic with the number of alleles per locus ranging from 2 to 9. All inbreds could be distinguished on the basis of RFLP analysis. Since several of

the inbreds were closely related on the basis of pedigree, RFLP analysis seems to be a useful method of identifying genetic differences and, perhaps, measuring genetic diversity in corn. Principle component analysis (PCA) of inbred RFLP allele frequency was effective in assigning lines to appropriate heterotic groups. PCA was also effective in identifying appropriate breeding groups for several inbreds of unknown parentage. This suggests RFLP analysis could be helpful in selecting parents for breeding research. In a related study, RFLP allele frequencies of the 27 inbreds were used to calculate measures of genetic relationships between the inbred lines and 4 inbred line testers. Each of the 27 inbreds were crossed to 1 or more of the testers and crosses were evaluated for grain yield. There was no statistically significant relationship between RFLP-based genetic distance measures and grain yield of the crosses. RFLP analysis was also conducted on a chromosome arm substitution series in a W22 background. Five versions of inbred W22, each containing a different segment on the distal 67% of the long arm of chromosome ten (10 L) were evaluated as lines per se and in crosses to several inbred testers for 11 quantitative traits.

Publications: 88/01 to 88/12

LEE, M., GODSHALK, E.B., LAMKEY, K.R. and WOODMAN, W.W. (1988). Molecular evaluation of combining ability in maize (Zea mays L.). (Accepted) Crop Sci.

LEE, M. (1989). Molecular genetic diversity among maize inbred lines: Taxonomic and plant breeding implications. In:
Development and Application of Molecular Markers to Problems in Plant Genetics. T. Helentjaris and B. Burr (eds.).

LEE, M., GODSHALK, E.B. and LAMKEY, K.R. (1988). Molecular marker analysis of chromosome segment substitutions in maize. Agronomy Abstracts 80:170.

GODSHALK, E.B., LEE, M. and LAMKEY, K.R. (1988). Restriction fragment length polymorphism analysis of maize single crosses. Agronomy Abstracts 80:81.

14.022 CRISO079226 GENETICS AND CYTOLOGY OF MAIZE

PETERSON P A; Agronomy; Iowa State University, Ames, IOWA 50011.

Proj. No.: IOW02381 Project Type: HATCH Agency ID: CSRS Period: O1 JUL 79 to 30 SEP 87

Objectives: Dissect and analyze genetically, several genes as model systems. Develop methods that will facilitate the isolation of specific DNA fragments for the analysis of their molecular structure. Obtain maize tissues in tissue culture in order to study gene interactions in an in vitro system. Elucidate gene interactions in hybrids of adapted lines with numerous accessions at the biochemical level. Relate these model interactions to hybrid performance identified in corn breeding programs. Elaborate, describe and define controlling and regulatory element systems in the maize genome. Explore the diversity of controlling element systems among the race of

maize. Develop and analyze model gene systems as they are applicable to the improvement of major crop types.

Approach: Genetic analysis will aid in uncovering novel variation, analyzing controlling element systems and derivatives. Tissue culture methods will be used to aid in the analysis of in vitro interaction. Electrophoretic and biochemical methods will be applied to the study of model enzyme systems. Gene cloning, sequencing and molecular analysis will be proved in collaboration with D. H. Sadler (West Germany).

Progress: 79/07 to 87/09. Mobile elements in maize. The Cy system has been in our populations for three decades. This was not possible to determine until a reporter allele (bz-rcy) was uncovered. With this allele it was found that the mutant a2m 668291, was caused by the induction of an rcy element into the A2 gene. Other mutants now have also been found to be Cy controlled. The Cy has been shown to have some relation to the mutator system. -- The mutant a-m(r) has been found to have a product that suppresses the functioning of an En element at the waxy locus. This is surprising since the a-m(r) allele has heretofore been considered a null form. This means there are many elements putting out products (unseen) that are suppressing the functioning of numerous mobile element functions. -- A miniature seed mutant has been discovered. It is cosegregating with the Uq element. This means that it is possible therefore, to rescue this allele providing the Uq element can be cloned.

Publications: 79/07 to 87/09

PETERSON, P.A. 1986. Mobile elements in maize: A force in evolutionary and plant breeding processes. Genetics Development and Evolution. Edited by J. Perry Gustafson, G. Ledyard Stebbins, and Francisco J. Ayala. Plenum Publishing Corp.

PETERSON, P.A. and SALAMINI, F. 1986. A
Search for Active Mobile Elements in the
Iowa Stiff-Stalk Synthetic Maize Population
and Some Derivatives. Maydica XXXI:163-172.

PETERSON, P.A. 1986. Mobile Elements in Maize and Effects on Gene Expression.

Gene Structure and Function in Higher Plants. Edited by G.M. Reddy and E.H. Coe, Jr. Oxford and IBH Publishing Company. New Delhi.

14.023 CRISO132859 INVESTIGATION OF THE MAIZE GENOME BY GENETIC, CYTOLOGICAL, AND MOLECULAR MEANS

PETERSON P A; Agronomy; Iowa State University, Ames, ${f IOWA}$ 50011.

Proj. No.: IOW02850 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: The major objective is to understand the genetics, the cytology, and the molecular biology of maize. It is proposed to tag various genes by transposons. To elaborate on the effect of transposons in corn breeding lines. To investigate gene interactions and relate these interactions into breeding lines

of corn. To elaborate on various transposon systems in the maize genome. To uncover additional transposons in an effort to determine their distribution in corn breeding lines. To search for, via transposons, disease resistant genes for their eventual cloning and isolation.

Approach: Genetic techniques will be used to uncover the transposon systems in maize. Collaboration with breeders will uncover the kind of variability generated in a maize line. The transposon tagging of disease resistant genes will be done with the aid and help of pathologists in several continents.

Progress: 88/01 to 88/12. Transposable genetic elements have uncovered considerable information about the maize genome. In addition to rescuing a large number of genes such as A1. C1, A2, C2. by transposon tagging, these elements have been instrumental in efforts to understand how genes function by affecting both codigenic and regulatory sequences. For example, new promoter effects have been uncovered. A number of genes have been analyzed as to insert content, and their system relationship has been established. A number of new inserts at the P locus are available. Some of the inserts at the C-I allele have affected their color suppressing potency. How can a C-I allele suppress color. A surprising finding is the extent of "additional baggage" that a gene transcript can sustain. A number of recovered exceptions show a changed phenotype that includes the reading of the insert as part of the transcript message. -- One, of the more significant aspects of transposable elements is their pervasiveness in corn-breeding populations. They appear to segregate strongly with the increase in frequency of favorable alleles. Along with this observation, the most frequent element, Uq, becomes activated even in lines that do not show an active element. These have been recovered as germinal events. In two cases, this activation has resulted in the induction of two developmental mutants, because they control the growth of kernels (size). They should be important for the isolation of genes controlling growth processes.

Publications: 88/01 to 88/12

SCHNABLE, P. S. and P. A. PETERSON. (1986). Distribution of Genetically Active Cy Transposable Elements Among Diverse Maize Lines. Maydica XXXI:59-81.

TACKE, E., ZS. SCHWARZ-SOMMER, P. A.
PETERSON, and H. SAEDLER. (1986). Molecular
Analysis of states of the A1 Locus of Zea
mays. Maydica XXXI:83-91.

PAZ-ARES, JAVIER, UDD WIENAND, PETER A.
PETERSON, and HEINZ SAEDLER. (1986).
Molecular cloning of the c locus Zea mays:
a locus regulating the anthocyanin pathway.
EMBO Journ. 5:829-833.

WIENAND, UDO, ULRIKE WEYDEMANN, URSULA NIESBACH-KLOSGEN, PETER A. PETERSON, and HEINZ SAEDLER. (1986). Molecular cloning of the c2 locus of Zea mays, the gene coding for chalcone synthase. Mol. Gen. Genet. 203:202-207. PETERSON PETER A. (1986). Mobile elements in maize: A force in evolutionary and plant breeding processes. Genetics Development and Evolution. Edited

by J. Perry Gustafson, G. Ledyard Stebbins, and Francisco J. Ayala. Plenum Publishing Corp.

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14.024 CRISO084781 METHODS OF CONTROLLING CORN INSECTS

GUTHRIE W D; RUSSELL W A; ERBACH D C; Entomology; Iowa State University, Ames, IOWA 50011

Proj. No.: IOWO2513 Project Type: STATE Agency ID: SAES Period: O1 JUL 86 to 30 JUN 91

Objectives: Develop methods for control of corn insects.

Approach: Beauveria bassiana will be applied to cornstalk residue and young plants in the spring. Incidences of an epizootic will be noted. The efficacy and compatibility of dual infections of Vairimorpha necatrix and Nosema sp. will be evaluated. Protease activity in the black cutworm midgut will be determined. The role of protease in activating or deactivating the protoxin of B. thuringiensis will be elucidated. Determine economic thresholds in corn and popcorn. The impact of conservation tillage on corn insects will be determined. Evaluate new insecticides for insect control. Develop application procedures which will reduce insecticide loss and environmental contamination. The impact on insect populations by growing corn in alternating rows with soybeans and corn grown with living multches will be determined. Corn, popcorn, and sorghum genotypes with resistance to both generations of European corn borers will be developed. Larval and adult behavior will be determined. Migration patterns of corn insects will be determined.

Progress: 88/01 to 88/12. Progress was made in developing genotypes of corn resistant to both generations of the European corn borer. Molecular markers (RFLP) were used in an attempt to pinpoint resistance genes on corn chromosomes. An undescribed microsporidium was isolated for the first time from adult European corn borers. Aqueous applications of Bacillus thuringiensis significantly reduced larval corn borers several days after application. Treating popcorn hybrids with Cerone (a plant growth hormone) slightly reduced 2nd generation ECB infestation in 3 of 8 hybrids tested but was not sufficient to give economic control. A dent corn hybrid resistant to 1st generation but susceptible to 2nd generation ECB was resistant when infested at mid-whorl, intermediate at late whorl, susceptible at tassel emergence, and highly susceptible at anthesis. Seed treatments were effective in reducing populations of first generation European corn borer larvae in corn. Effectiveness was dependent on plant variety, tillage, and seed quality. Seed quality was also related to performance of soil applied insecticides as manifested by plant germination and plant heights. Populations of ECB were affected by

tillage. Overall, lower numbers of larval tunnels were found in no till plots as compared to those in plow, chisel, and till plant plots. Populations appeared to be related to plant maturity at time of moth flight.

Publications: 88/01 to 88/12

GUTHRIE, W. D., F. A. HASKINS, H. J. GORZ. (1988). Relationship of European corn borer resistance in sorghum to HCN-p and DIMBOA content in leaf and sheath-collar tissue. J. Agricul. Entomol. 5(1):21-28.

NYHUS, K. A., W. A. RUSSELL, and W. D. GUTHRIE. (1988). Response of two maize synthetics to recurrent selection for resistance to first-generation European corn borer (Lepidoptera: Pyralidae) and diplodia stalk rot. J. Econ. Entomol.

SAJAP, A. S., and L. C. LEWIS. (1988).
Effects of the microsporidium Nosema
pyrausta (Microsporida: Nosematidae) on the
egg parasitoid Trichogramma nubilale
(Hymenoptera: Trichogrammatidae). J.
Invertebr. Pathol. 52:294-300.

JARVIS, J. L. (1988). Damage by European corn borer (Lepidoptera: Pyralidae) to popcorn kernels compared with damage to kernels of dent corn and sweet corn. J. Econ. Entomol. 81:1214-1216.

JARVIS, J. L. and W. D. GUTHRIE. (1988).
Effect of first generation European corn
borer on yield and plant height of popcorn.
J. Agric. Entomol. 5:179-183.

14.025 CRISO131898 GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOODS

GLATZ B A; Food Technology; Iowa State
University, Ames, IOWA 50011.
Proj. No.: IOW02827 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 87 to 30 JUN 92

Objectives: To improve strains of Propionibacterium important in the dairy industry and in propionic acid production. To understand the genetic organization of this and of other Gram-positive organisms. To develop means of gene transfer among these organisms and other Gram-positive organisms. To identify and characterize important genetic determinants in these organisms.

Approach: Plasmids native to propionibacteria will be isolated and characterized. Genes carried on plasmids will be sought, and characterized when found. Conjugations using conjugative plasmids from other Gram-positive organisms or from the propionibacteria will be established. Transformation of DNA into protoplasts or whole cells will be performed. Bacteriophage specific for propionibacteria will be sought. Mutants altered in important traits will be constructed.

Progress: 88/01 to 88/12. The goal of the research is to investigate the genetics of the propionibacteria, which are important industrial organisms. Screening of the culture collection of 119 strains of propionibacteria for the presence of plasmid DNA has been completed. Twenty strains were found to contain

plasmids, and at least 10 unique plasmids were identified in these strains. Seven plasmids were partially characterized by restriction endonuclease analysis, and restriction maps were constructed for four of these. Hybridization studies were conducted to determine the relationships among the seven plasmids that were partially characterized. Five of these plasmids were cured from their respective strains by chemical treatment, and all plasmidcarrying strains and cured derivatives were checked for antibiotic resistances, carbohydrate fermentations, and bacteriocine production. Three plasmid-associated traits have been identified: lactose fermentation and a possible cell-surface component on one plasmid, and cell clumping on another plasmid. The culture collection has been screened for the presence of temperate and/or inducible bacteriophage and bacteriocin production, and samples of rumen fluid, Swiss cheese whey, silage, and lake water have been tested for the presence of lytic phage or other inhibitory components. One strain, recently isolated from Swiss cheese, appears to contain a defective bacteriophage.

Publications: 88/01 to 88/12
GLATZ, B.A. and ANDERSON, K.I. (1988).
 Isolation and characterization of mutants
 of Propionibacterium strains. J. Dairy Sci.
 71: 1769-1776.

14.026 CRISO034260 TRANSPOSABLE ELEMENTS IN MUTATOR ACTIVITY IN MAIZE

MORRIS D W; Genetics; Iowa State University, Ames, IOWA 50011. Proj. No.: IOW02707 Project Type: CRGO Agency ID: CRGO Period: 01 AUG 84 to 31 JUL 88

Objectives: PROJ 8400352. Mutator in maize is responsible for increasing mutation frequencies by up to 50-fold. Transposable elements have been implicated in its activity, and one such element, Mul, is associated with some Mutator-induced mutations. We will define more clearly the role played by Mul and other transposable elements in Mutator activity.

Approach: We will use molecular hybridization and cloning techniques to: characterize Mul-like elements from a number of Mutator and putative Mutator lines of maize; continue studying the effects of inbreeding and outcrossing on Mul copy number and transposition; characterize a number of Mutator-induced mutations (both stable and unstable) at known loci, such as Sh and Wx, for which cloned genes are available; characterize putative Mutator-induced mutations in chlorpolast DNAs.

Progress: 88/01 to 88/12. 1. The putative progenitor alleles and 4 Mu-induced sh (shrunken) mutants were characterized by restriction mapping. All Mu-induced mutants had a Mu related insertion sequence in the 5' regulatory region and one had an additional insertion in the 3' region. 2. Loss of mutator germ line activity in some inbred Mu stocks is

accompanied by the loss of the ability of Mu elements to transpose and the methylation of restriction sites, which are not cut by methylation-sensitive enzymes (Mu-element modification). 3. A cytoplasmically inherited mutant found in a Mu cross was shown to lack the thylakoid cytochrome f/b-563 complex. 4. Twenty lines of maize, from many countries, were screened for the presence of Mul-homologous sequences. All lines contained 2-9 copies of sequences homologous to Mul internal and terminal portions. Two to four unmodified Mul-like elements were found in plants and tissue cultures of the inbred A188. 5. Some stocks of Mu-induced mutable al mutants seem to be segregating for a single autonomous element. The Mu elements in the plants from mutable seeds in these stocks had DNA with less methylation at Hinf1 sights than plants from stable seeds. Tests, also showed that Mul , Mul.4 and Mu3 do not act as an autonomous element in these lines. 6. Somatic mutability was found in endosperm tissue cultures of Mu-induced mutants of wx (waxy) and bz (bronze). Revertant sectors, however, can not be propagated in culture.

Publications: 88/01 to 88/12 BENNETZEN, J. L., FRACASSO, R. P., MORRIS, D. W., ROBERTSON, D. S., SKOGEN-HAGENSON, M. J. (1987). Mol. Gen. Genet. 208:57-62. MASTERSON, R. V., BIAGI, K., WHEELER, J. G., STADLER, J., MORRIS, D. W. (1988). An embryogenic cell line of maize from A188 (Minnesota) contains Mul-like elements. Plant Mol. Biol. 10:273-279. ROBERTSON, D. S., MORRIS, D. W., STINARD, P. S., ROTH, B. A. (1988). Germ line and somatic Mutator activity: are they functionally related. in Plant Transposable Elements ed. Oliver Nelson, Plenum Press, New York. pp. 17-42. MOURAD, G., POLACCO, M., ROBERTSON, D., SKOGEN-HAGENSON, M. J., MORRIS, D. W. (1988). A maternally inherited mutant of Zea mays L. lacks the cytochrome f/b563 complex. Submitted to Curr. Genetics. ROTH, B. A. (1987). Robertson's Mutator system in maize: Studies on the regulation of activity and the prevalence of Mul-homologous DNA sequences in diverse lines of maize. Ph.D. Thesis (ISU). ANDERSON, J. M. (1987). The molecular characterization of mutations induced in Mutator lines at the Bronzel and Shrunken loci in maize. Ph.D. Thesis (ISU).

14.027 CRISO089219 GENE STRUCTURE AND EXPRESSION IN PLANTS

MORRIS D W; Genetics; Iowa State University, Ames, IOWA 50011. Proj. No.: IOW02602 Project Type: HATCH

Agency ID: CSRS Period: 01 DEC 82 to 30 SEP 87

Objectives: The primary objectives of this research program are to study gene structure and expression in agriculturally important plants. Although recombinant DNA techniques have been applied with success to study chloroplast DNA, plant nuclear DNA, due to its large size and complexity, offfers a more

difficult challenge. Date, very few plant genes are available to study their structure and function. We shall be using recombinant DNA techniques to isolate and characterize structural genes from established genomic libraries prepared from corn nuclear DNA. We envisage that this knowledge will eventually contribute to a broader basis on which the genetic modification of plant genomes can be developed into a useful additional method in plant breeding for generatig phenotypic variation in proven genotypes.

Approach: DNA: DNA hybridization methods will be used to identify and isolate specific genes from corn genomic libraries. Structural genes will be analyzed by restricution endonuclease mapping and DNA sequencing. Concurrently, we shall be expoiting yeast cloning techniques as a potentially powerful system in which metabolic genes may be isolated from corn genomic librariesby functional complementation of yeast deficiencies, and the expression of structural components of plant genes may be studied.

Progress: 82/12 to 86/12. This project has now been totally assimilated into Project Number IOW02707, "The role of transposable elements in Mutator in maize". Funds for project 2602 enabled much ground work to be accomplished for this latter project: the characterization of DNA probes to be used for gene characterization and cloning (examples of such genes being shrunken, bronze, and waxy and Mutator-induced mutants of these genes), and the adaptation of specialized techniques such as Southern blot hybridizations, and so forth.

Publications: 82/12 to 86/12 ROBERTSON, D.S., STINARD, P., WHEELER, J. and MORRIS, D.W. Genetic and molecular studies on germinal and somatic instability in Mu-induced aleurone mutants of maize. Plant Genetics: ICN-UCLA Symp. Molec. Cell. Biol., New Series, Volume 35. BENNETZEN, J.L., FRACASSO, R.P., MORRIS, D.W., ROBERTSON, D.S. and SKOGEN-HAGENSON, M.J. Concomitant loss of Mu 1 transposition

and Mutator activity in maize. Molec. Gen.

14.028 CRIS0090332 GENETIC ANALYSES OF THE MUTATOR SYSTEM OF MAIZE

Genet. in the press.

ROBERTSON D S; Genetics; Iowa State University, Ames, IOWA 50011. Proj. No.: IOW02623 Project Type: HATCH Agency ID: CSRS Period: O1 MAR 89 to 30 SEP 93

Objectives: To generate Mutator-tagged mutants that can be used for isolating genes for quantitative traits and genes with commercial potential (e.g., amylose extender (ae) mutants). To isolate the master gene (regulator) that controls the Mutator system. To study the role of the Mutator system in generating deletions and other chromosomal aberrations. To clone the Y1 gene, which regulates the carotenoid pathway in corn with the goal of transferring it to rice; thereby increasing the nutritional value of rice and

alleviating wide-spread suffering and death, which afflicts the children of many rice eating people. To determine the reversion frequency of several Mutator- induced mutants. To determine the stages in the life cycle of the plant when transposition of Mu elements occurs.

Approach: The procedures will involve the informed utilization of stocks that have been generated over the years. This system has been studied. Techniques that have proven successful by maize geneticist and which have been used for the past 15 years to study the Mu system will continue to be utilized.

Progress: 88/01 to 88/12. A better understanding of the following aspects of the Mutator system (Mu) of maize bas been obtained: 1. Mu germinal activity can be lost through inbreeding and outcrossing. Inbred loss stocks are inactivators of active Mu lines while outcross loss lines are not. 2. Mutability can be restored to many mutable Mu-induced mutants that have lost their mutability by crossing to germinally active Mu stocks. 3. The loss of somatic mutability of a mutable Mu-induced mutant is followed by the loss of germinal activity within 1 or 2 generations. 4. Stocks of Mu-induced mutable mutants have been developed that seem to segregate for a single regulator element of somatic mutability. These stocks will be helpful in isolating the elusive regulator element of the Mu system. 5. Mu induced deletion involving the yg2, Bf1 and al sh2 loci have been found and characterized. 6. The Mu system can induce mutations in the male and female gametophytes and during early development of the embryo. (Previously, Mu was known to have premeiotic activity and also possibly meiotic). 7. Reversion frequencies have been established for several Mu-induced mutants. 8. A dominant putative Mu-induced amylose extender mutant (Ae*-5180) has been studied genetically and biochemically. This mutant increases the normal level of amylose 25% to 70% in kernels with 1, 2, or 3 copies of Ae-5180. This mutant could be useful in the commercial production of amylose starch.

Publications: 88/01 to 88/12

LAMKEY, K. R. , HALLAUER, A. R. , and ROBERTSON, O. S. (1988). Contribution of the long arm of chromosome 10 to the total heterosis observed in five maize hybrids. Crop Sci. 28:896-901.

ROBERTSON, O.S., MORRIS, O.W., STINARO, P.S., and ROTH, B.A. (1988). Germ line and somatic Mutator activity: Are they functionally related. Basic Life Sciences, Vol. 47.

ROBERTSON, D.S., and STINARO, P.S. (1987). Genetic evidence of Mutator-induced deletions in the short arm of chromosome 9 of maize. Genetics 115:353-361.

ROBERTSON, O.S. (1986). Genetic studies on the loss of Mu Mutator activity in maize. Genetics 113:765-773.

ROBERTSON, O.S., STINARD, P.S., WHEELER, J.G. and MORRIS, D.D. 1985. Genetic and molecular studies on germinal and somatic instability in Mutator-induced aleurone mutants of maize. Plant Genetics, ed. Freeling, M. Alan R. Liss, Inc., N. Y.

ROBERTSON, D.S. (1985). A possible technique for isolating genic DNA for quantitative traits in plants. J. Theor. Biol. 117:1-10. ROBERTSON, O.S. (1985). Differential activity of the maize mutator Mu at different loci and in different cell lineages. Mol. Gen. Genet. 200:9:13.

14.029 CRISO135914 ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE

ROBERTSON O S; MYERS A M; LEE M; Genetics; Iowa State University, Ames, IOWA 50011.

Proj. No.: IOW02870 Project Type: CRGO Agency IO: CRGO Period: O1 MAY 88 to 30 APR 90

Objectives: Proj. 8800435. To combine molecular and genetic approaches to isolate genes affecting the quantitatively inherited trait of kernel size.

Approach: Transposon tagging with the Mutator transposable element system will be used to facilitate the isolation of putative quantitative genes for cloning. Cloned genes will be mapped by utilizing traditional genetic techniques. Restriction fragment length polymorphisms (RFLPs) will also be used to place quantitative genes for kernel size to specific region of the genome. Finding that these two techniques have identified the same regions of the genome would strongly suggest that a putative quantitative gene for kernel size resides there and has been cloned.

14.030 CRISO138155 ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE

ROBERTSON D S; MYERS A M; LEE M; Genetics; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: IOW02916 Project Type: CRG0 Agency IO: CRG0 Period: 01 MAY 89 to 30 APR 90

Objectives: To combine molecular and genetic approaches to isolate genes affecting the quantitatively inherited trait of kernal size. Proj. 8902420.

Approach: Transposon tagging with the Mutator transposable element system will be used to facilitate the isolation of putative quantitative genes for cloning. Cloned genes will be mapped by utilizing traditional genetic techniques. Restriction fragment length polymorphisms (RFLPs) will also be used to place quantitative genes for kernel size to specific regions of the genome. Finding that these two techniques have identified the same regions of the genome would strongly suggest that a putative quantitative gene for kernel size resides there and has been cloned.

14.031 CRISO135867
GENETIC/MOLECULAR CHARACTERIZATION OF THE
MUTATOR-RELATED CY TRANSPOSABLE ELEMENT SYSTEM
OF MAIZE

SCHNABLE P S; Genetics; Iowa State University, Ames, IOWA 50011.

Proj. No.: IDW02882 Project Type: HATCH Agency ID: CSRS Period: 07 SEP 88 to 30 JUN 93

Objectives: The primary objective of this project is to clone and analyze the Mutator-related Cy transposable element. This element will be invaluable in resolving the complex Mutator phenomenon. Secondary objectives include the development and use of Cy/rcy stocks suitable for tagging experiments, development of a bz-rcy-based system of generating RFLPs on given chromosome arms, and molecular characterization of rcy transposition and rcy-mediated chromosome rearrangements.

Approach: Both molecular and genetic approaches will be used to isolate the Cy element. First, rcy and other Mu elements will be used as molecular probes against genomic digests of plants with and without Cy. The genetic approach will be to trap Cy at ascorable gene for which a gene probe already exists.

Progress: 88/07 to 88/12. An isolation plot was used to generate a population of approximately 400,000 individuals. This population is being screened for mutations at the A locus. Putative transposon insertion mutations are being isolated at a rate of approximately 1/10,000. These need to be tested to determine whether they are heritable and if so whether any of them arose via a Cy insertion. Isolation of a confirmed Cy-induced mutation at the A locus will facilitate the cloning of this transposon. Preliminary genetic evidence has been obtained which suggests that excision of the rcy: Mu7 transposon from the Bz locus is correlated with its subsequent re-insertion elsewhere in the genome. This finding supports the feasibility of enriched gene tagging with Mu elements. It also has implications regarding the model of Mu transposition.

Publications: 88/07 to 88/12
No publications reported this period.

14.032 CRISO004941 CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS

POLLAK E; Statistics; Iowa State University, Ames, IOWA 50011.

Proj. No.: IOW01448 Project Type: STATE Agency IO: SAES Period: 01 JUL 59 to 01 JAN 99

Objectives: Study of population genetics, with particular reference to balanced polymorphisms maintained by natural selection occurring in human and other species. Consultation on mathematical problems arising from workers in genetics.

Approach: Procedure will consist partly of the examination of theoretical models and will be partly in cooperation with individuals who have collected or are collecting data on genetic populations.

Progress: 88/01 to 88/12. E. Pollak provided assistance to Dr. A. R. Hallauer of the Department of Agronomy, who asked a question concerning covariances between relatives when a population originally has a Hardy-Weinberg structure and successive generations are produced by self fertilization. Let FS(subscript n) be the mean of a full sib family resulting from a cross between two plants that are produced after n generations of selfing. It was verified that the covariance between FS(subscript 0) and FS(subscript 4) is equal to the covariance between full sib offspring of individuals of generation O. Assistance was also provided to Mr. Brad Hedges, a student in the Department of Agronomy. He was faced with the problem of calculating what family size is large enough so that, if there are two possible sets of underlying frequencies of K types of offspring of a cross, the probabilities of the two kinds of misclassification are each 0.025. Professor C. P. Cox of the Department of Statistics and E. Pollak collaborated in solving this problem. Previously, the solution was known only if there are two types of offspring.

Publications: 88/01 to 88/12

JUNG, Y. C., ROTHSCHILD, M. F., FLANAGAN, M.
P., POLLAK, E. and WARNER, C. M. Genetic
variability between two breeds based on
restriction fragment length polymorphisms
(RFLPs) of major histocompatability complex
class I genes in the pig.

14.033 CRISO095517 BIOCHEMISTRY OF GENETIC SYSTEMS

REECK G R; Biochemistry; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KANO0563 Project Type: STATE
Agency ID: SAES Period: 01 JUL 85 to 30 JUN 88

Objectives: To characterize further the structure, particularly the domain structure, of members of the HMG-1 family of chromosomal proteins, to investigate the functions of the HMG-1 family of proteins, to isolate a full-length cDNA coding for the corn inhibitor of trypsin and activated Hageman factor, to assess the level of similarities and the evolutionary relatedness of families of proteins, including plant storage proteins.

Approach: The chromosomal protein, HMG-1, will be purified from fetal or newborn calf thymus by extraction of chromatin with 0.35 M NaCl, chromatography on DNA cellulose columns, phospho-cellulose columns, or a column containing immobilized histone H5, polyclonal antibodies against calf thymus HMG-1 and mouse monoclonal antibodies will be used to deplete extracts of HMG-1 and HMG-2, a cDNA library will be made in corn, comparisons of amino acid sequences will be performed using computerized methods.

Progress: 85/07 to 88/06. We have found that by altering reaction conditions, endoproteinase GluC will release a variety of fragments from the nonhistone chromosomal protein HMG-1. The availability of these fragments, on a preparative scale, will allow testing of the contributions of the various domains of the protein to the interactions it makes with DNA, histones, and chromain. A cDNA clone that encodes the 12K corn inhibitor has been isolated and completely sequenced. This ill allow site-directed mutagenesis of the inhibitor to establish the mechanisms of its interactions with insect trypsins and amylases that it inhibits. Wheat proteins have been isolated by reversed-phase HPLC that specifically inhibit the digestive amylases of the rice weevil. Sequencing of the inhibitors (now underway) will allow isolation of cDNA clones that encode them. cDNA clones have been isolated and sequenced for two rice endosperm proteins--glutelin and LMW-globulin. Rice gluetelin is a homolog of pea legumin, and the LMW-globulin is a homolog of wheat protease and amylase inhibitors. The availability of the cDNA clones will allow expression of th proteins in yeast and site directed mutagenesis aimed at improving the nutritional quality of rice protein and an understanding of the basis of inhibition by the LMW-globulin protein.

Publications: 85/07 to 88/06

PAN, S.J. and REECK, G.R. 1988. Isolation and characterization of rice alpha-globulin. Cereal Chemistry 65:316-319.

PAN, S. 1988. Amino acid analysis of total protein and four major protein families of rice endosperm and partial sequence of a cDNA clone encoding rice LMW-globulin. Ph.D. disseertion.

WANG, C. 1988. Molecular biology of rice glutelin. Ph.D. dissertation.

14.034 CRISO095292 GENETICS AND PHYSIOLOGY OF FUSARIA

LESLIE J F; Plant Pathology; Kansas State University, Manhattan, KANSAS 66506.

Proj. No.: KANO0547 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 90

Objectives: Characterize natural populations of Fusarium moniliforme with respect to mating groups, mating types, vegetative incompatibility groups, meiotic mutants and DNA restriction fragment length polymorphisms, and measure correlations of these traits with pathogenicity. Define the overall regulation of nitrogen catabolism in Fusarium roseum 'Graminearum' and test both naturally occurring variants and mutants induced in the laboratory for nitrogen catabolic activity, pathogenicity and the production of mycotoxins.

Approach: F. moniliforme isolates from corn and sorghum will be subdivided on the basis of their race, mating-type and vegetative incompatibility group. Representatives of each class will be tested for pathogenicity in a corn seedling system, and for the presence of meiotic mutants and DNA restriction fragment length polymorphisms. Nitrogen catabolic

activity in mutant and wild-type strains of F. roseum 'Graminearum' will be measured by dry weight of cultures grown in shake flasks, linear growth rates in race tubes, resistance to common nitrogen analogs and defects in enzymes such as nitrate reductase, glutamate dehydrogenase and glutamine synthetase. Mutants defective in nitrogen catabolism will be tested for pathogenicity in a corn seedling system.

Progress: 88/01 to 88/12. We have completed classical genetic studies of nitrate metabolism in F. moniliforme. We mapped all seven nit mutants with respect to each other and tested nitrate reductase levels of these mutants (no activity was detected). nit1 and nit3 mutants were recovered most frequently, but the relative frequencies with which the mutants occurred could be altered by changing the nitrogen source in the minimal chlorate medium. Different strains throw chlorate sectors at different frequencies. These frequency differences are heritable as a quantitative trait and are consistent with a transposable element as the cause for the sectoring phenomenon. A series of five crn mutants (chlorate-resistant, utilize nitrate) were also characterized. All of these mutants had detectable nitrate reductase activity, and two of them appear to be allelic with different nit mutants. The other three loci may be involved in regulation or in nitrate uptake. We have continued to characterize populations of F. moniliforme using vegetative compatibility as our measure of variability. At least 40 different vegetative compatibility groups (VCGs) have now been identified. In general F. moniliforme populations are highly variable with respect to this trait. We have also described a novel phenomenon - termed "heterokaryon self-incompatibility" - which can give false negatives in complementation tests; in our studies this trait is under the control of a single nuclear gene.

Publications: 88/01 to 88/12

KLITTICH, C.J.R. and LESLIE, J.F. 1988. Nitrate reduction mutants of Fusarium moniliforme (Gibberella fujikuroi). Genetics 118:417-423.

KLITTICH, C.J.R., CORRELL, J.C. and LESLIE, J.F. 1988. Inheritance of sectoring frequency in Fusarium moniliforme (Gibberella fujikuroi). Experimental Mycology 12:289-294.

KLITTICH, C.J.R. and LESLIE, J.F. 1988.

Multi-well plates for complementation tests
of Fusarium. Fungal Genetics Newsletter
34:21-22.

KLITTICH, C.J.R. and LESLIE, J.F. 1988. Chlorate-resistant, nitrate-utilizing mutants of Fusarium moniliforme (Gibberella fujikuroi). Journal of General Microbiology (in press). 14.035 CRISO076599
BIOLOGY, EPIDEMIOLOGY & CONTROL OF VIRUSES &
MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF
CORN

LOMMEL S; Plant Pathology; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KANOOOO9 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 82 to 30 SEP 88

Objectives: Identify and characterize viruses, spiroplasmas and MLO's occurring in corn and sorghum in the United States and to develop methodology suitable for virus detection in epidemiological studies.

Approach: conduct statewide surveys; collect tissue samples for analyses. Produce viral antisera and develop serological test procedures. Establish host range differential for virus identification. Characterize biological and serological properties of viral agents. Establish field plots and inoculate at different plant growth stages and evaluate for symptomatology and yield. Screen crop genotypes for sources of resistance to viral agents; study effect of cultural practices on disease epidemiology.

Progress: 78/07 to 88/09. The complete genome of maize chlorotic mottle virus (MCMV) has been cloned and sequenced. The genome organization and replicative strategy of the virus has been determined. In collaboration with Dr. R.C. Nutter at Oklahoma State University, the in vitro synthesis of various gene products is being determined. An infectious transcription system for MCMV is currently being developed to perform precise structural and functional mutations.

Publications: 78/07 to 88/09
LOMMEL, S.A., KENDALL, T.L., SIU, N.F. and
NUTTER, R.C. 1988. cDNA cloning and in
vitro translation of the maize chlorotic
mottle virus genome. J. Gen. Virol.
(Submitted).

NUTTER, R.C., SHEETS, K., PANGANIBAN, L.C. and LOMMEL, S.A. 1989. The complete nucleotide sequence of the maize chlorotic mottle virus genome. Virology (In preparation).

14.036 CRISO093861 STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE

ZIMMER E A; Biochemistry; Louisiana State University, Baton Rouge, **LOUISIANA** 70803. Proj. No.: LABO2414 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 89

Objectives: To examine DNA variation in maize and teosinite ribosomal genes. To determine which variable characters are stably inherited and which are unstably inherited. To investigate mechanisms of unstable inheritance. To develop stably-inherited variants as markers of foreign germplasm introduction in monocot crop plants.

Approach: Molecular Biology and Classical Genetic Studies. Nucleic acids will be isolated from various tissues of corn and teosinte. Variation in DNA sequence and structure for a particular class of nuclear genes, those coding for robosomal RNA, will be assessed with enzymatic, electrophoretic and isotopic analysis. The mode of inheritance of the variant features identified will be assessed in crosses among corn lines and in interspecific hybrids between corn and teosinte.

Progress: 88/01 to 88/12. During the 1987-1988 period, we extended our studies of ribosomal gene variation as it relates to systematics of the grass family, Poaceae, and further developed our studies of differential ribosomal gene expression in maize and teosintes. Technically, we have added automated DNA ampliciation and assays of differential RNA production to our repertoire for addressing these questions. Automated DNA amplification is of use to both the systematics and gene expression projects; the RNA assays are of most use to the second, but were developed from information gained with the "comparative sequencing for systematics" project. Specifically, the systematics project is being extended to include two primitive general of grasses, Diarrhena and Brachyelytrum, whose placement is relatively unclear and to rye, Secale cereale, which was inadvertently excluded from the original study (Hamby and Zimmer, 1988). We also will begin sequencing rRNA from teosintes tripsacums for use in both systematics and expression work. These projects are being conducted by Honors' undergraduates in my laboratory. Mr. Hamby, my senior graduate student, has begun to sequence, via the polymerase chain reaction, cp rDNA from the same species studied by cytoplasmic rRNA.

Publications: 88/01 to 88/12

HAMBY, R.K., SIMS, L.E., ISSEL, L.E. and ZIMMER, E.A. 1988. Direct ribosomal RNA sequencing: Optimization of extraction and sequencing methods for work with higher plants. Technical report, Plant Molecular Biology Reporter, 6:179-197.

HAMBY, R.K. and ZIMMER, E.A. 1988. Ribosomal RNA sequences for inferring phylogeny within the grass family (Poaceae). Plant Systematics and Evolution, 160: 29-37.

ZIMMER, E.A., JUPE, E.R. and WALBOT, V.E. 1988. Ribosomal gene structure, variation and inheritance in maize and its ancestors. Genetics, 120: 1125-1136.

ZIMMER, E.A., HAMBY, R.K., ARNOLD, M.L., LDBLANC, D.A. and THERIOT. 1989. Ribosomal RNA phylogenies and flowering plant evolution. In: The Hierarchy of Life. Proceedings of the 70th Nobel Foundation Symposium, Elsevier Press, in press.

SACHDEV, V., JUPE, E.R. and ZIMMER, E.A. 1988. A survey of ribosomal gene methylation in Tripsacum. Maize Genetics Cooperative Newsletter, 62: 28-29.

JUPE, E.R. and ZIMMER, E.A. 1988. DNA methylation, chromatin structure and expression of maize ribosomal RNA genes. Invited platform presentation, workshop on Chromatin Structure and Gene Expression.

HAMBY, R.K. and ZIMMER, E.A. 1988. A molecular perspective on monocot origins. Genome 30s: p 78.

14.037 CRISO135660 ANALYSIS OF GENES CONTROLLING KEY FLORAL DIFFERENCES BETWEEN TEOSINTE AND MAIZE

GALINAT W C; Suburban Exper Station; University of Massachusetts, Waltham,

MASSACHUSETTS 02154.

Proj. No.: MAS-8801056 Project Type: CRG0 Agency ID: CRG0 Period: 01 JUN 88 to 31 MAY 92

Objectives: PROJ. 8801056. Determine the background factors necessary for penetrance and stabilization of expression by the key trait genes; determine precisely where the basis key trait genes separating teosinte and maize are located, their interaction with each other and with their genomic background; determine the nature of clustered female spikes in teosinte in contrast with solitary ones (ears) in maize as a possible key trait difference, how it is inherited and where the controlling gene(s) are located, how it interacts with other traditionally recognized key traits and their background; characterize the relationship of the essential chromosome 4 complexes of teosinte and maize to each other and to their homeologs (Tr7 and Tr13) from Tripsacum.

Approach: The key-trait genes (tr,pd) will be backcrossed into primitive backgrounds and their interaction with specific genes having primitive effects will be evaluated. The key-trait genes will be located by use of A-B translocations and by use of cross-mapping with Tripsacum derivatives. Isogenic backgrounds developed by backcrossing and selection will be based on the inbreds W23 and A158. The vascular anatomy of the female rachis will be compared to phenotypic stability of the key traits. The over-all flower size will be analysed as a balanced system in contrast with independent changes in component size within the flower as an unbalancing threat.

14.038 OOO7368 BIOCHEMICAL AND DEVELOPMENTAL GENETICS OF HIGHER PLANTS

GENGENBACH B G; Agronomy & Plant Genetics; University of Minnesota, St Paul, **MINNESOTA** 55108.

Proj. No.: MIN-13-032 Project Type: STATE Agency ID: SAES Period: 01 JUL 89 to 30 JUN 94

Objectives: Determine gene-enzyme relationships and isolate genes regulating amino acid biosynthesis, amyloplast development, and herbicide tolerance. Characterize mitochondrial transmission genetics and mutants. Develop tissue cultures of malting barley.

Approach: Specific genes will be isolated by molecular biology methods from normal and mutant genotypes to determine their role in

amino acid biosynthesis, amyloplast development and herbicide tolerance. Progeny from plants containing heterogeneous mitochondrial genomes will be analyzed for molecular and phenotypic traits. Plants will be regenerated from barley tissue cultures and evaluated for malting quality, herbicide tolerance and agronomic traits.

Progress: 88/01 to 88/12. DNA and plant markers were used to prove that cytoplasmically-inherited mitochondrial (mtDNA) in corn can segregate through the female parent. Molecular analyses of individual maternal plants identified the relative proportions of two mtDNA arrangements known to be associated with T cytoplasm male sterility/disease susceptibility or male fertility/resistance. These analyses predicted the frequencies of corresponding contrasting plant phenotypes in the progeny. Rearrangements in a specific repeated mtDNA sequence adjacent to the atp9 gene were identified in four fertile mutants obtained from sterile S cytoplasm corn. S cytoplasm mtDNA was shown to have two atpA genes located in different regions of the genome. The genome organization of one atpA gene changed and the new version was amplified during a few generations of seed propagation indicating that mtDNA variation is established quickly. The cytochrome oxidase III gene from soybean mtDNA was isolated and sequenced. MtDNA polymorphisms among soybean lines derived from the Lincoln source of Mandarin germplasm were identified. Barley genotypes screened in the field and greenhouse for reaction to the herbicide imazethapyr exhibited differences, but no genotype was resistant. Selection for resistance in tissue cultures of Morex and comparisons of acetolactate synthase (herbicide target site) in sensitive and more tolerant genotypes have been initiated.

Publications: 88/01 to 88/12

RINES, H.W., GENGENBACH, B.G., BOYLAN, K.L. and STOREY, K.K. 1988. Mitochondrial DNA diversity in oat cultivars and species. Crop Sci. 28:171-176.

PRING, D.R., GENGENBACH, B.G. and WISE, R.P. 1988. Recombination is associated with polymorphism of the mitochondrial genomes of maize and sorghum. Phil. Trans. R. Soc. Lond. B 319:187-198.

WANG, Y. 1988. Molecular analysis of mutants from maize cms-S fertile revertants. Ph.D. Thesis. Univ. Minnesota. St. Paul. 111p.

Thesis. Univ. Minnesota, St. Paul. 111p. BURTON, J.D., STOLTENBERG, D.E., GRONWALD, J.W., SOMERS, D.A., GENGENBACH, B.G.

and WYSE, D.L. 1988. Inhibition of acetyl-coenzyme A carboxylase by sethoxydim and haloxyfop. Plant Physiol, 86s:111.

WANG, Y. and GENGENBACH, B.G. 1988. Unique mtDNA restriction fragment in an S cytoplasm fertile revertant line retaining the S1 and S2 plasmids. Miaze Genet. Coop. Newslett. 62:102-103.

GENGENBACH, B.G. 1988. Maternal segregation for a mtDNA deletion in maize. Genome 30 (supp.):315.

WANG, Y. and GENGENBACH, B.G. 1988. Fertile revertants of Scms maize have unique mtDNA fragments. Genome 30(supp.):315.

14.039 CYTOGENETICS IN PLANT IMPROVEMENT

CRISO006177

PHILLIPS R L; Agronomy & Plant Genetics; University of Minnesota, St Paul, **MINNESOTA** 55108.

Proj. No.: MIN-13-022 Project Type: HATCH Agency IO: CSRS Period: O1 OCT 87 to 30 SEP 92

Objectives: Develop somatic cell genetics systems for crop species; Design molecular genetic methods for transferring important traits; Elucidate and apply molecular and developmental cytogenetic information; develop genetic selection procedures for protein quality improvement.

Approach: Molecular and classical cytogenetic methods will be employed to test the basis of tissue culture-induced genetic variation; use molecular genetic markers to determine the chromosomal distribution of genes controlling agronomic traits; understand molecular biological basis of kernel growth; & design selection procedures for obtaining strains with improved protein quality.

Progress: 88/01 to 88/12. The gene regulating expression of a methionine-rich corn storage protein led to increased lysine levels when introduced into certain genetic backgrounds. A lysine + threonine laboratory seedling screening procedure was shown to detect altered ratios of methionine to lysine in corn kernels. DNA amplification in whole endosperms was documented by flow cytometry to achieve 3X the base DNA level. Differential replication of parts of the genome was shown not to occur. Ribosomal ONA (rONA) in endosperm nucleoli was found to be less methylated than contiguous, non-nucleolar rONA and represents specific rONA sequences. Transposable element (Ac) activation via the tissue culture process might be due to the activation of one of the $7\,$ to 8 cryptic sequences shown to be present; one DNA segment co-segregates with Ac activity and a ONA methylation change is present in one case. Activation of a Spm element via tissue culture occurred in one instance. Molecular genetic probes were identified that distinguishes Tripsacum chromosomes in crosses with highly heterogeneous maize populations. A B73 genetic male-sterile system using duplicate-deficient chromosomes led to the production of an all male-sterile line (90 plants). Oat rONA was localized to only the three satellited chromosomes. Chromosome 2 was shown to carry a distinct rONA sequence. Little polymorphism for rDNA was shown for oat while related wild oat species are highly polymorphic.

Publications: 88/01 to 88/12

ARMSTRONG, C.L. and PHILLIPS, R.L. 1988.
Genetic and cytogenetic variation in plants regenerated from organogenic and friable, embryogentic tissue cultures of maize. Crop Sci. 28:363-369.

LEE, M., GEAOELMANN, J.L. and PHILLIPS, R.L. 1988. Agronomic evaluation in inbred lines derived from tissue cultures of maize. Theor. Appl. Genet. 75:841-849.

BENZION, G. and PHILLIPS, R.L. 1988.
Cytogenetic stability of maize tissue
cultures: A cell line pedigree analysis.
Genome 30:318-325.

PHILLIPS, R.L., MCMULLEN, M.O., ENOMOTO, S. and RUBENSTEIN, I. 1988. Ribosomal ONA in maize. p. 201-214. In J.P. Gustafson and R. Appels (ed.) Chromosome Structure and Function: Impact of New Concepts.

KOWLES, R.V. and PHILLIPS, R.L. 1988. Endosperm development in maize. Intl. Rev. Cytol. 112:97-136.

LEE, M.O. and PHILLIPS, R.L. 1988. Chromosomal basis of somaclonal variation. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39:413-437.

PHILLIPS, R.L. and PESCHKE, V.M. 1988.
Discovery of Ac activity among progeny of tissue culture derived maize plants. Intl. Symp. Plant Transposable Elements, Madison, WI p. 305-315.

14.040 CRISO084575 MOLECULAR MAPPING OF GENES IN CORN

RUBENSTEIN I; Genetics & Cell Biology; University of Minnesota, St Paul, **MINNESOTA** 55108.

Proj. No.: MIN-72-050 Project Type: HATCH Agency IO: CSRS Period: O1 JUL 85 to 30 SEP 88

Objectives: Develop hybridization techniques able to determine the chromosomal location of maize genes. Determine the chromosomal location of the genes of the zein multigene family.

Approach: Use cONA probes to detect differences in hybridization and restriction fragment length polymorphisms of genomic blots prepared from the ONA of normal maize plants vs. the ONA of aneuploid plants. Use the techniques devised to map cONA probes to map the chromosomal location of zein genes.

Progress: 85/01 to 88/09. The major accomplishments during the grant period are: 1. Determination of the sequence of the spacer region of the 17/25S rRNA gene. This publication represents the first complete sequence of the rRNA spacer region of a cereal. The spacer contains 10 subrepeats of 200 base pairs. They are located near the apparent promoter site of RNA synthesis initiation and may play an important role in the control of rRNA transcrition. 2. Determination of the 5S gene number heterogeneity in the Illinois Protein corn lines. The 5S ribosomal gene copy numbers were found to be unrelated to the protein containing the 17/26\$ rONA gene copy number, or the rRNA transcript levels in the Illinois Protein lines. The physical techniques used to determine these results will be useful to the physical mapping of genes in corn.

Publications: 85/01 to 88/09

MCMULLEN, M.D., HUNTER, B., PHILLIPS, R.L. and REBENSTEIN, I. 1986. The structure of the maize ribosomal DNA spacer region. Nucleic AcidsRes. 14:4953-4968.

NEWMAN, S.M. and RUBENSTEIN, I. 1988. 5S rONA gene number heterogeneity in maize inbreds selected for protein content divergence.

(MS in preparation).

14.041 TISSUE CULTURE GENETIC SYSTEMS

CRISO096427

PHILLIPS R L; RINES H W; Office of Research Admin.; University of Minnesota, St Paul, **MINNESOTA** 55108.

Proj. No.: MIN-8500192 Project Type: CRG0 Agency ID: CRG0 Period: O1 AUG 85 to 31 JUL 89

Objectives: Proj 8500192. Further document the occurrence of chromosomal and genetic variability in cell cultures and regenerated plants of oats and maize, test unifying hypothesis accounting for both chromosome breakage and new mutants, develop means of modifying cell genetic behavior, and evaluate possible applications of culture instability.

Approach: Experiments will test: a) the role of late replicating DNA and chromosome breakage in generating variability, b) the release of transposable elements during the tissue culture process, c) organogenic versus embryogenic cultures relative to stability and origin of plantlets, and d) the occurrence of gene transfer by chromosome exchange induced by the tissue culture process.

Progress: 88/01 to 88/12. Transposable genetic element (Ac) activated by the maize tissue culture process were studied at the molecular level. Cryptic Ac sequences were shown to be present in parental lines. A 10 kb Bgl II DNA fragment homologous to Ac cosegregated with the new Ac activity. Altered DNA methylation was detected in one Ac-positive line. Approximately 700 tests for Spm activation via tissue culture revealed two cases of newly induced Spm activity. Agronomic evaluation of 269 oat lines derived from tissue culture-regenerated plants revealed both positive and negative variations for eight traits: height, heading date, grain yield, bundle weight, seed number, 100 seed weight, flag leaf area, and percent protein. Five lines showed significantly greater yield than the controls and 22 lines yielded significantly less Isoelectric focusing revealed banding pattern variation in avenin proteins among some of the lines. Fifteen haploid oat plants were recovered using embyro rescue following application of maize pollen to about 3000 emasculated oat florets. Cytological analysis of early stage embryonic and endosperm cells at division revealed lagging chromosomes, chromosomes not associated with mitotic figures, and numerous micronuclei. These observations support the suggestion that oat/maize hybrid zygotes are produced followed by elimination of the maize chromosomes during early embryo development.

Publications: 88/01 to 88/12

ARMSTRONG, C.L. and PHILLIPS, R.L. 1988.

Genetic and cytogenetic variation in plants regenerated from organgenic and friable, embryogenic tissue cultures of maize. Crop Sci. 28:363-369.

LEE, M., GEADELMANN, J.L. and PHILLIPS, R.L. 1988. Agronomic evaluation of inbred lines derived from tissue cultures of maize. Theor. Appl. Genet. 75:841-849.

BENZION, G. and PHILLIPS, R.L. 1988.
Cytogenetic stability of maize tissue
cultures: A cell line pedigree analysis.
Genome 30:318-325.

PHILLIPS, R.L., SOMERS, D.A. and HIBBERD, K.A. 1988. Cell/tissue culture and in vitro manipulation. In Corn and Corn Improvement (ed G.F. Sprague). Am. Soc.

Agron., Madison, WI (In press).
LEE, M.D. and PHILLIPS, R.L. 1988.
Chromosomal basis of somaclonal variation.
Ann. Rev. Plant Physiol. Plant Mol. Biol.
38:413-437.

PHILLIPS, R.L. and PESCHKE, V.M. 1988.
Discovery of Ac activity among progeny of tissue culture-derived maize plants. Intl. Symp. Plant Transposable Elements, Madison, WI p. 305-315.

SOMERS, D.A., PHILLIPS, R.L. and RINES, H.W. 1988. Corn and oat tissue cultures and genetic variation in regenerated plants. FFTC Symp. on Cell and Tissue Culture in Field Crop Improvement. Tsukuba, Japan (In press).

14.042 CRISO132664 AMYOPLAST DIFFERENTIATION IN DEVELOPING MAIZE ENDOSPERM

JONES R J; GENGENBACH B G; Office of Research; 1919 University Avenue, St Paul, MINNESOTA 55104.

Proj. No.: MINR-8701093 Project Type: CRG0 Agency ID: CRG0 Period: O1 JUL 87 to 30 JUN 90

Objectives: PROJ. 8701093. To compare maize endosperm amyloplast DNA with chloroplast DNA, To identify genes transcribed in amyloplasts and determine whether transcripts are specific to amyloplast biogenesis, To analyze the developmental relationships between amyloplast biogenesis, amyloplast gene expression, starch accumulation, and transcription of specific nuclear genes during endosperm tissue differentiation.

Approach: DNA from amyloplasts will be compared to clones of the complete chloroplast genome by hybridization and restriction mapping. Plastid-specific RNA from endosperm will be hybrididized to the plastid genome clones to determine the spectrum of transcripts present and whether novel transcripts can be identified. Genes for novel transcripts will be mapped, cloned and characterized in detail. Cellular aspects of amyloplast differentiation will be investigated by light and electron microscopy.

Progress: 88/01 to 88/12. Cosmid clones which cover the entire plastid genome were used in Southern hybridization experiments to confirm that corn endosperm contains plastid DNA. Hybridization patterns of total DNA from endosperm tissue were identical to those of total DNA of leaves indicating no changes in organization of the plastid genome during endosperm development. The ratio of plastid DNA

to nuclear ribosomal DNA in endosperm cell total DNA extracts increased during the 8- to 16-day post-pollination period. This indicates that the plastid DNA increases more than the nuclear DNA which also is known to increase during endosperm development. The pattern of plastid DNA accumulation was similar in inbreds A188 and B7; however, the ratio of plastid DNA to nuclear DNA was higher throughout endosperm development in A188. Plastid DNA accumulation n reciprocal F1 endosperms is being analyzed. Nuclease protection and northern hybridization experiments have indicated that most if not all of the plastid genome is transcribed in endosperm plastids. The number of transcript species that hybridize to a given plastid probe and the accumulation of most transcripts is lower in endosperm than in leaf tissue. Unprocessed or uniquely processed transcripts from some ribosomal protein genes, however, do accumulate preferentially during endosperm development. These transcripts and their accumulation patterns are currently being characterized.

Publications: 88/01 to 88/12

MCCULLDUGH, A.J. and GENGENBACH, B.G. 1988.

Plastid DNA in developing endosperm. Maize
Genet. Coop. Newslett. 62:102.

MCCULLDUGH, A.J. and GENGENBACH, B.G. 1988.

Molecular biology of maize amyloplast
development. Genome 30(supp.):460.

14.043 CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS

CDE E H; BECKETT J B; Agricultural Research Service; Agricultural Research Service, Columbia, **MISSOURI** 65211.

Proj. No.: 3802-20040-008-00D

Project Type: INHDUSE Agency ID: ARS Period: O2 APR 80 to O1 JAN 86

Objectives: Broaden basic genetic and cytogenetic knowledge in corn to increase our understanding of mechanisms of gene action and chromosomal behavior and establish more reliable bases and more efficient means for crop improvement.

Approach: Analyze mechanisms of nuclear and non-nuclear inheritance, including contributions of specific genes, chromosomes, and organelles to the growth, structure, form, photosynthetic and storage efficiency, and biochemical constituents. Produce a full set of B-A translocations with more thorough coverage of the genome; converge to selected inbred lines and produce homozygous strains. Converge trisomics to selected inbreds. Analyze the effects of specific chromosomal segments on measurable characters. Characterize mechanisms controlling pairing and exchange of chromosomes, and of non-disjunction. Test the feasibility of diverging genomes artificially to the point of forming stable polyploid hybrids. Define methods by which duplicated genetic segments can be derived, toward designed alteration of plant efficiency and constituents.

Progress: 80/04 to 85/12. Comprehensive information on genetics of corn assembled and made available to research workers worldwide through the Maize Genetics Cooperation News Letter, genetic lists, maps, & coordination of mapping. Development of the embryo, plant body, leaf, tassel & ear progresses by cells attaining limited destinies, rather than by unlimited cells advancing & producing limited ones. Tools for analysis & manipulation of chromosome segments, B-A translocations, enhanced by addition of new versions. characterization, localization, & production of uniform strains in homozygous form. Dther tools, including tertiary trisomics, trisomics, inversions, & telocentrics, have been produced, & characterizations of them & their effects have been advanced. Accumulation & fixation of aneuploidy in autotetraploids has been shown to be a major contributor to inbreeding depression. Recognized existence of differential pairing units. Extensive mapping of the genome has been carried out, & genetic factors for plant form, for polyphenolic compounds, for chloroplast thylakoid proteins, & for meiotic control have been characterized genetically. Nuclear genes affecting the chloroplasts & mitochondria with heritable alterations have been shown to have background-specific functional effects & to influence plant form & development in dramatic ways; sorting of mixed organelles of both types has been proposed as an explanation for sectoring, & molecular & cytological evidence has been found supporting this explanation.

Publications: 80/04 to 85/12
NO PUBLICATIONS REPORTED THIS PERIOD.

14.044 GENETIC MECHANISMS IN CORN

CRISO140738

CDE E H; BECKETT J B; DDYLE G G; Cereal Genetics Research Unit; Agricultural Research Service, Columbia, **MISSOURI** 65211.
Proj. No.: 3622-21220-001-00D

Project Type: INHDUSE Agency ID: ARS Period: O1 JAN 86 to 31 DEC 90

Objectives: Develop basic genetic and cytogenetic knowledge and techniques applicable to the analysis, evaluation and modification of nuclear and organellar genetic material of corn plants.

Approach: Ascertain genetic mechanisms of determination of functions; conduct inheritance analysis of flavonoid (polyphenolice) pathway, developmental control, and regulation of biosynthesis. For manipulation of the genome, produce and analyze B-A translocations and telocentric chromosomes. Determine mechanisms of chromosome pairing, crossing over and disjunction, in A and B chromosomes, translocations, aneuploids and inversions. Chromosome segment contributions to morphology. combining ability, through dosage studies and substitutions of segments will be performed. The processes and basis of chromosome divergence, and of genetic variation in homozygous geneotypes will be determined. Define cellular destinies and critical genetic

control points in development. Mitochondrial and chloroplast genetics, including organelle-nucleus interactions will be measured.

Progress: 88/01 to 88/12. Screened materials for telocentric chromosomes, and derived new occurrences; 16 telocentrics representing 9 arms on 8 chromosomes have been obtained. Characterizations are in progress on the cytological and genetic behavior of these telocentrics. Telo6La has been characterized and the behavior has been summarized and evaluated; manuscript has been submitted. Advanced project to introduce segments from tropical variety Antigua into Corn Belt germplasm. Made crosses to produce new B-A translocations to enhance the set, and tested new translocations. Continued backcrossing and derivation of homozygous B-A translocations. Designed and initiated experiments to map reciprocal translocations in the long arm of chromosome 10, and other segments, through use of nondisjunction of the B-10L18 chromosome in combinations, and through other nondisjunction systems. Developed materials for study of dosage effects of B and R on enzymes of flavonoid biosynthesis, including a flavanone 3-hydroxylase. Carried out testcrossing for allelism of 46 new recessive and 5 new dominant leaf-striping mutants vs. 8 previously defined mutants, and initiated analysis of the tests. Renewed stocks of tester materials and translocations. Advanced linkage analyses.

Publications: 88/01 to 88/12

BECKETT, J.B. 1989. Cytogenetic, genetic and plant breeding applications of B-A translocations in maize. In: Tsuchiya, T., and Gupta, P.K., Eds., Chromosome Engr. in Plant Genetics & Breeding. Elsevier, Amsterdam. Accepted Feb. 18, 198.

COE, E.H., THOMPSON, D. and WALBOT, V. 1988. Phenotypes mediated by the Iojap genotype in maize. Am. J. Bot. 75:634-644.

WENDEL, J.F., GOODMAN, M.M., STUBER, C.W. and BECKETT, J.B. 1988. New isozyme systems for maize (Zea mays L.): Aconitate hydratase, adenylate kinase, NADH dehydrogenase, and shikimate dehydrogenase. Biochem. Genetics 26:421-445.

14.045 CRISO049430 GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS

STEINER W M M; Parasite-predator Bio & Ecol Bio Control of Insects Lab; Agricultural Research Service, Columbia, MISSOURI 65211. Proj. No.: 3622-24000-006-00D

Project Type: INHOUSE Agency ID: ARS Period: 01 OCT 84 to 30 SEP 89

Objectives: Genetically map and characterize chromosomal, morphological, DNA, and allozyme variants of selected entomophagous insects and determine their physiological, developmental and behavioral significance and their potential for utilization in bioengineering programs to enhance entomophageefficiency.

Approach: Populations of selected entomophages will be assayed using classical gene- tic techniques and restriction enzyme (DNA) technology to establish gene-tically typed isolines. These will be used to establish linkage maps and study genome structure in the context of predator-parasite/prey models. About 80 % of effort will be devoted to the model offered by Heliothis zea and its entomophages with 20% of effort reserved to develop genetic know- ledge of other models. Selected variants will also be biochemically char- acterized and their distributions in natural populations determined. Lab- oratory tests of physiological, developmental and behavior response under stresses posed by pesticides, temperature and desiccation will reveal whichphenotypes can enhance natural population fitness characteristics. Methods of transferring desirable traits will be developed.

Progress: 88/01 to 88/12. Selection for insecticide resistance in females of the parasitoid Microplitis croceipes was initiated to determine how amenable this parasite of Heliothis spp. is for development of insecticide resistant varieties. After 5 generations of inbreeding, two of five lines demonstrated an increase in LD values, going from 0.82 ug fenvalerate (in 0.5 ul acetone topical application) to a 16-fold increase at 13.12 ug with an accompanying increase in number of males being produced and a decline in line fertility. Selection ceased at the 6th round when the lines were lost due to the increased selection pressure. The observed selection response is a typical one seen for braconid wasps, and suggests this is the upper limit to which the M. croceipes genome may respond to insecticide selection. In other studies, three species of nabids were found to segregate for allozyme variation at an esterase locus (EST-1!) and an adenylate kinase locus (ADK-3). These were not in genetic equilibrium and reduction in the numbers of heterozygotes in nature suggests strong population subdivision. These loci may serve a diagnostic function to differentiate closely related nabid species.

Publications: 88/01 to 88/12
GRASELA, J.J., STEINER, W.W.M. and MARSTON,
N.L. 1988. Genetic differences at two
allozyme loci in midwest populations of
three species of Nabidae. Comp. Biochem.

Physiol. 90B:427-431.

STEINER, W.W.M. 1988. Electrophoretic techniques for the genetic study of aphids In, A.K. Minks and P. Harrewijn (Eds.) "Aphids, Their Biology, Natural Enemies and Control", Vol. B, Elsevier Sci. Publ., Amsterdam, pp. 135-143.

14.046 CRISO141825 CHROMOSOME SEGMENTAL EFFECTS IN CORN

COE E H; BECKETT J B; DOYLE G G; Agricultural Exper. Station; University of Missouri, Columbia, MISSOURI 65211.

Proj. No.: 0101-21020-009-07S

Project Type: COOPERATIVE AGREE.

Agency ID: ARS Period: O1 OCT 86 to 31 MAY 89

Objectives: Employ the efficient cytogenetic tools and materials available in corn to carry out analyses of the genetic content, effects and interactions of segments comprising the genome, across a selected group of elite inbred lines.

Approach: Define locations in the genome of variants affecting growth, development, form and function, and of polymorphisms in the DNA. Assemble and compile information on genome mapping. Columbia, MO, Tucker Hall; Rm 303; BL-1; July, 1984. E. H. Coe, J. B. Beckett, G. G. Doyle, D. A. Hoisington, M. G. Neuffer, K. J. Newton.

Progress: 88/01 to 88/12. Advanced backcrossing of 17 B-A translocations and r-x1 to the 5th backcross level in 4 elite inbred lines of corn. Began increase of seed supplies of these at the 5th backcross level, and of testers appropriate to them, targeting to announce release as soon as possible. Carried out evaluation of choices of strains for chromosome-segment substitutions and defined the appropriate choices for substitutions to be initiated. Designed experiments toward study of agronomic traits at the diploid level segment-by-segment, and toward evaluation of stalk strength, kernel row number and tassel branch number in 1 vs. 2 vs. 3-dose conditions segment-by-segment. Incorporated two new B-A translocations on chromosome 2, short arm, into the backcrossing program. Screened source materials for telocentrics and isolated 16 telocentrics for 9 different chromosome arms. Characterizations of the cytological and genetic behavior of the telocentrics are being conducted.

Publications: 88/01 to 88/12
 JOHNSON, S.S. and COE, E.H., JR. 1988.
 Estimation of genetic effects of specific
 genomic segments in maize. Agro. Abst. p.
169.

14.047 CRISO033919 GENETIC CONTROL OF LEAF LESION FORMATION IN MAIZE

NEUFFER M G; WALBOT V; Agronomy; University of Missouri, Columbia, **MISSOURI** 65211.

Proj. No.: MO-OO276-3 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 82 to 31 AUG 85

Objectives: Proj. No. 8200437. Investigate the genetics of disease lesion mimic mutation in maize.

Approach: To identify and compare dominant and recessive lesion mutants from spontaneous and mutagen induction sources. Comparisons to be made in different genetic backgrounds such as W23 which appears to enhance lesion formation and Mo2OW which suppresses lesions, and environmental conditions such as variations in temperature, humidity, light, and stress. Characterizations will be made by locating mutants to chromosome using the Twx method for dominants and the B-A translocation method for

recessives. Placements on the linkage map will be made with 3 point linkage tests. Transmission, viability, and heritability will be determined by analysis of progenies from outcrosses and selfs.

Progress: 85/01 to 85/12. Seventeen dominant and 8 recessive lesion mutant genes have been identified. Of these, 12 of the dominant and 2 of the recessives have been located to chromosome arm. The only alleles so far identified are 2 pairs of recessives. Fifth backcross progeny for 11 dominant mutants are available as well as hybrid and triple-cross hybrid materials. Lesion expression/enlargement has been studied in various non-green tissue. Lesions do not form on Les1 tissue that is senescing, not fully expanded, etiolated, white as in wt1 as the herbicide Sandoz 9789, or kept at 30 C. Lesions do form in Les1 tissue that is hemizygous, heterozygous, or homozygous for Les1; is white due to wd1; has reduced chlorophyll due to Oy1; or has been grown continuously at 20 C or shifted from 30 C to 20 C. Les1 appears to be cell autonomous for all stages of lesion propagation. In leaf chimeras lesions form only on Les1 tissue, not in adjacent Les1+ tissue. Light has been found to be a strong promoter, or perhaps a required factor, for necrotic lesion formation in Les1 plants. A possible increase of a 67 Kd protein has been observed in Les1 homozygotes upon shifting from 30 to 20 C, which induces lesion formation. The protein increase is observed before lesions have formed.

Publications: 85/01 to 85/12
HOISINGTON, D.A., WALBOT, V. and NEUFFER,
M.G. Sectorial loss of Les1. Maize Genet.
Coop. Newsletter 57:160. (1983).
RAY, N. and WALBOT, V. Marking pens can cause
lesions in Les mutants. Maize Genet. Coop.
Newsletter 58:190. (1984).
HOISINGTON, D.A. Linkage studies of lesion
and necrotic mutants. Maize Genet. Coop.
Newsletter 58:82-84. (1984).
WALBOT, V., HOISINGTON, D.A. and NEUFFER,
M.G. Disease Lesion Mimics in Maize. II.
Chimeric analysis of lesion initiation and
enlargement of Les1. In preparation.

14.048 CRISO034232 NUCLEAR GENES IN CORN WHICH REGULATE PROTEINS FOR CHLOROPLAST MEMBRANES

MILES D; Biological Sciences; University of Missouri, Columbia, MISSOURI 65211.

Proj. No.: MO-8400511 Project Type: CRGO Agency ID: CRGO Period: 15 SEP 84 to 31 MAR 87

Objectives: PROJ 8400511. Our goal is to better understand nuclear gene regulation of chloroplast development and of photosynthesis. A large amount of information is available concerning the biochemistry of photosynthesis. Chloroplast genes for photosynthetic proteins are being rapidly described; however, there are few examples of nuclear genes regulating specific chloroplast proteins. We plan to examine nuclear regulation by describing nuclear genes which encode protein components of the chloroplast membranes.

Approach: Our objectives will be to induce photosynthesis mutants in maize with the transporable DNA element, mutator, Mul. These mutants will be characterized to the specific reaction of photosynthesis and the proteins altered. Restriction fragments of nuclear DNA containing Mul will be isolated and correlated with the mutant phenotype. These will be cloned using standard methods of molecular cloning. The wild-type gene sequence will be recovered from a genomic library and/or the normal transcript will be isolated by hybrid selection. The protein products of these gene clones can be characterized and correlated with similar well characterized mutations in maize. Verified nuclear photosynthesis genes (structural or regulatory) which are cloned can be sequenced.

Progress: 88/01 to 88/12. This award has allow our laboratory to continue our research using the transposon Mutator to induce photosynthesis mutants in maize. We have now isolated and characterized 15 different mutant nuclear genes in corn. These newly uncovered genes have given us new information of the make-up photosystem I reaction centers and the role of plastoquinone in the photosystem II reaction center. We have identified a specific 4.4 kilobase fragment of DNA in mutant hcf3-Mu which co-segregates with the photosystem II mutant phenotype. This fragment of DNA is now being cloned and characterized. This approach as allow us access to cloning a gene which regulated photosynthesis without knowing the nature of the gene product. This research is continuing under a new USDA/CSRC research grant.

Publications: 88/01 to 88/12

MILES, D. and METZ, J.G. 1985. The Role of Nuclear Genes of Maize in Chloroplast Development. In Plant Genetics, UCLA-Keystone Symposium, M. Freeling (ed.), A.R. Liss, New York, pp. 585-597.

BARKAN, A., MILES, D. and TAYLOR, W.C. 1986. Chloroplast Gene Expression in Nuclear, Photosynthetic Mutants of Maize, MBO Journal, 5:1421-1427.

KOBAYASHI, H., BOGORAD, L. and MILES, D. 1987. Nuclear Gene-Regulated Expression of Chloroplast Genes for Coupling Factor One in Maize Plant Physiology 85:757-767.

COOK, W.B. and MILES, D. 1988. Transposon mutagenesis of nuclear photosynthetic genes in Zea mays. Photosynthesis Research, in press.

14.049 CRISO132170 ANALYSIS OF GENETIC RECOMBINATION IN MAIZE POPULATIONS USING MOLECULAR MARKERS

STASWICK P; COMPTON W A; THOMAS-COMPTON M A; Agronomy; University of Nebraska, Lincoln, NEBRASKA 68583.

Proj. No.: NEB-12-169 Project Type: STATE Agency ID: SAES Period: O1 JUL 87 to 30 JUN 90

Objectives: 1. Analysis of recombination rates in maize populations utilizing molecular markers. 2. Each population will be subdivided on the basis of divergent recombination rate,

intermated within each category and the extent of divergence determined. 3. Determination of the quantity of heterochromatic nuclear materials in families shown to differ in recombination rates. 4. Based on information derived from these studies, we hope to develop a methodology of synthesizing populations with divergent rates of recombination for further study.

Approach: The following populations will be used in this study: (1) CBMEX 3 (2) CBCAR 5 (3) NS O (4) B73×N7A F2 & other F2 generations from elite single crosses in which RFLPs can be readily monitored. Frequencies of alleles at a number of enzymeloci have already been determined in these materials. Ele ctrophoretic techniques will be used to identify genotypes homozygous for contrasting allozymes & these will be crossed to produce double heterozygotes. The progenies will be selfed & rates of recombination de-termined. Families or their selfed progenitors with high & low recombi- nation rates will be compared at meiosis for frequencies of knobs, B chromosomes, & other prominent heterochromatic segments. Flow cytometric techniques will also be used to determine DNA content in these families

Progress: 88/01 to 88/12. This study uses restriction fragment length polymorphisms (RFLP) as gene markers to detect differences in rates of recombination in various F(subscript 2) maize families. The original maize lines and RFLP markers came from Native Plants, Inc. (NPI). Laboratory--Methodology for detecting RFLP's was adapted for our facilities. Purified stocks of marker DNA were made from the sample plasmids sent from NPI. All of the important inbred corn lines in this study were characterized for their RFLP pseudoalleles. Field--F(subscript 1) seed was produced in the greenhouse in the winter and again in the summer, F(subscript 2) families were obtained in the summer by selfing plants that came from seed produced in the greenhouse the previous winter. It is necessary to produce the seed under different environmental conditions as this may affect rates of recombination.

Publications: 88/01 to 88/12

No publications reported this period.

14.050 CRISO097231 TISSUE-SPECIFIC GENE REGULATION IN MAIZE

KLEIN A; Spaulding Life Science Bldg; University of New Hampshire, Durham, **NEW HAMPSHIRE** 03824.

Proj. No.: NH-8502624 Project Type: CRG0 Agency ID: CRG0 Period: 30 SEP 85 to 30 JUN 88

Objectives: Proj 8502624. A Ds-controlling element mutation at the bronze-1 locus (bz) in maize, exhibits an abnormal developmental and tissue-specific pattern of gene expression. I have cloned this allele, bz-m4 Derivative 6856. Analysis of this mutant will help elucidate the molecular organization of cis-acting regulatory sequences controlling tissue-specific gene expression.

Approach: The organization of bz-m4 Derivative 6856 will be determined by i) restriction mapping of the cloned allele and ii) sequencing of the rearrangement breakpoints of the insertion. The levels and structures of transcripts of the mutant and wild-type alleles will be compared to determine the mechanisms by which the Ds insertion modifies developmental regulation of bz.

Progress: 87/01 to 87/12. The mutation bz-m4 Derivative 6856 alters the temporal and tissue specific expression of the bronze gene product (Dooner CSHSQB 45:457, 1981). This mutation resulted from the insertion of a novel 6.7 kb Ds transposable element at the locus bronze (Klein et. al. submitted). Two (2 kb) Ds elements are at either ends of the insertion and flank a partial duplication of 3' flanking sequences from the bz locus. The entire complex transposon was inserted 36 bp downstream from the wild-type mRNA cap site. The 6.7 kb insertion in bz-m4 displaces the normal promoter for the gene. Leila Paje-Manalo in my laboratory has isolated poly A-enriched mRNA from immature seeds of both wild-type (Bz-McC) and mutant (bz-m4) material. The transcript in bz-m4 is less abundant but is similar in size to that of the wild-type mRNA (1.9 kb). Two interpretations of this result are plausible: Transcription of the mutant allele is intiated from the normal promoter; the 6.7 kb transposable element is spliced from the message resulting in a functional mRNA. Changes in gene expression result from an subtle enhancer effect of the transposable element. Alternatively, one of the Ds elements provides a new promoter resulting in the production of message similar in size to the wild-type mRNA.

Publications: 87/01 to 87/12
KUHN, E. and KLEIN, A.S. Expression of the
 developmental mutant bz-M4 Derivative 6856
 in maize seedlings. Phytochemistry
 26:3159-3162, 1987.

14.051 CRISCO99483 EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIDIZING POPULATIONS

Objectives: To assess, using molecular markers, the extent of genetic differences between two pheromone strains of European Corn Borer. To characterize patterns of variation and covariation of localities where both strains occur, so that the extent of genetic exchange between strains can be understood.

Approach: Two sorts of variation will be examined: variation in soluble enzymes (proteins) using starch gels and histochemical strains; variation in restriction sites of mitochondrial DNA--detected by hybridization of digests of total DNA from individual moths with P-labeled pure ECB mtDNA.

Progress: 86/08 to 87/09. Using standard techniques we have isolated and purified mitochondrial DNA (mtDNA) from laboratory strains of European corn borer (ECB). Total DNA from individual moths has been isolated, digested with restriction endonucleases, run on agarose gels, transferred to nitrocellulose, and probed with 32P-labeled pure mtDNA. We have surveyed restriction fragment patterns for 30 enzymes. Of these, 11 have no recognition sites (do not cut the mtDNA). Included in this group are several enzymes which recognize four-base sequences (HaeIII - GGCC, HhaI - GCGC). These results suggest that the ECB mtDNA molecule is extremely A+T-rich. Using double digests, we have constructed a restriction site map for a single ECB strain for nine enzymes. We are in the process of comparing fragment patterns (site maps) between pheromone strains (Z and E) and between univoltine and bivoltine populations in New York (strains provided by W. Roelofs). We have also obtained (from R. Carde) males from a presumably mixed (Z and E) population, which were attracted to different pheromone blends. Results from these comparisons will indicate whether the pheromone strains and ecotypes found in the United States are distinct genetic entities.

Publications: 86/08 to 87/09
NO PUBLICATIONS REPORTED THIS PERIOD.

14.052 CRISO084829 CODING AND REGULATION OF MITOCHONDRIAL GENES

FOX T D; Genetics Development; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-1864O1 Project Type: HATCH Agency ID: CSRS Period: 13 MAR 89 to 30 SEP 92

Objectives: In all eucaryotes, the expression of mitochondrial genes within the organelle must be coordinated with the expression of nuclear genes. The long term goal of this project is to understand the molecular mechanisms by which mitochondrial gene expression is regulated. This proposal is to study nuclear genes that specifically control expression of the Saccharomyces cerevisiae mitochondrial genes encoding cytochrome oxidase subunits II (coxII) and III (coxIII) at the level of translation.

Approach: Determine the mechanisms by which translation of the mitochondrially coded mRNAs for coxIII and coxII is activated by nuclear genes. The key goal here is to identify the target molecules with which the known positive regulatory proteins interact. The action of the nuclear genes on coxIII translation will be studied by: a) Precisely mapping the site(s) of action for the nuclear genes PET494, PET54 and PET122 in the 5'-leader of the coxIII mRNA, using a new procedure for mitochondrial transformation to study the in vivo effect of in vitro-generated mutations. b) Selecting second site suppressors of mutations in the coxIII mRNA 5'-leader and in the three nuclear genes. c) Purifying the products of the three nuclear genes, to allow in vitro studies of protein-RNA binding. Determine how the nuclear genes PET494, PET54, PET122 and PET111 are

regulated.

Progress: 88/01 to 88/12. We discovered that a pBR322-derived plasmid carrying only the mitochondrial gene coding coxII (oxi1) could stably transform mitochondria in a strain totally lacking endogenous mtDNA (rhoo). Like the mtDNA sequences retained in natural rhostrains, the plasmid DNA in our transformants, including pBR322 sequences, was reiterated into concatemers whose size was indistinguishable from wild-type mtDNA. This "synthetic rho-" mtDNA was replicated and efficiently transmitted to mitotic progeny despite the absence of any pre-existing mtDNA in the host strain. oxil genetic information in these synthetic rho- strains could be expressed in diploids either after "marker rescue" by double recombination with rho+ mtDNA carrying an oxi1 point mutation, or in trans during the growth of diploids heteroplasmic for both the plasmid-derived oxi1 sequences and rho+ mtDNA deleted for oxi1. A key advantage of our approach to mitochondrial transformation is that the initial identification of low frequency transformants does not depend upon function of the mtDNA being introduced. With respect to mitochondrial functions, the transformants were phenotypically identical to the rhoo host strain. Mitochondrial transformants were detected by a "marker rescue" assay dependent only upon double recombination between a small portion of the introduced DNA and known point mutations in a rho+ partner. This procedure will readily allow introduction and detection of altered and possibly defective mitochondrial genes.

Publications: 88/01 to 88/12

CDSTANZD, M.C., SEAVER, E.C., MARYKWAS, D.L. and FDX, T.D. 1988. Multiple nuclear gene products are specifically required to activate translation of a single mitochondrial mRNA. IN: Genetics of Translation; New Approaches.

FDX, T.D., CDSTANZD, M.C., STRICK, C.A., MARYKWAS, D.L., SEAVER, E.C. and RDSENTHAL, J.K. 1988. Translational regulation of mitochondrial gene expression by nuclear genes of S.cerevisiae. Phil.Trans.Royal Soc. Lond.B 319:97-105.

CDSTANZD, M.C. and FDX, T.D. 1988. Specific translational activation by nuclear gene products occurs in the 5'-untranslated leader of a yeast mitochondrial mRNA. Proc. Natl. Acad. Sci. USA 85:2677-2681.

FDX, T.D., SANFDRD, J.C. and MCMULLIN, T.W. 1988. Plasmids can stably transform yeast mitochondria lacking endogenous mtDNA. Proc. Natl. Acad. Sci. USA 85:7288-7282.

CDSTANZO, M.C. and FDX, T.D. 1988. Transformation of yeast by agitation with glass beads. Genetics 120:667-670.

MARYKWAS, D.L. and FDX, T.D. 1988. Control of the Saccharomyces cerevisiae regulatory gene PET494: transcriptional repression of glucose and translational induction of oxygen. Mol. Cell. Biol., in press.

14.053 CRISO097066 CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF DS ELEMENT

DELLAPDRTA S L; Cold Spring Harbor Laboratory; P D Box 100, Cold Spring Harbor, **NEW YORK** 11724.

Proj. No.: NYR-8502679 Project Type: CRGD Agency ID: CRGD Period: 01 SEP 85 to 01 SEP 87

Objectives: Proj 8502679. Isolation and characterization of Germinal mutations of wx-ml: stable mutations of wx-ml will be isolated in field experiments during 1985 at both CSHL and a winter nursery. These mutations will grow in the Spring, 1986 for molecular analysis. Stock construction for isolating unstable wx-ml mutations: we are unable to recover unstable wx-ml mutations in Year 1 until we have constructed the proper genetic stocks. This summer we have moved the proper markers into the wx-ml material and will use our winter nursery to obtain the stocks needed for these experiments during Year 2.

Approach: Isolation and characterization of unstable wx-ml mutations: we expect to recover several states of wx-ml from our field experiments during the summer, 1986. Completion of the characterization of stable wx-ml mutations: in Year 2 we will have completed our molecular characterization of stable germinal mutations isolated in Year 1.

14.054 CRISO141444 ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE FUNCTION AND REGULATION

SISCD P H; Agricultural Research Service, Raleigh, **NORTH CAROLINA** 27607. Proj. No.: 6645-22000-002-00D

Project Type: INHDUSE Agency ID: ARS Period: 27 MAR 86 to 27 MAR 91

Objectives: For corn genes: isolate genomic clones of different alleles; determine DNA sequences; isolate native protein products; determine regulatory and structural regions of genes; investigate functional differences between alleles.

Approach: Transposable elements will be used to tag genes for isolation of genomic clones. Bacterial expression vectors will produce a partial protein sequence, which will then be used to make antibodies for isolation of the native protein; differences between DNA and protein sequences of alleles will be determined.

Progress: 88/01 to 88/12. Stocks for transposon tagging were put into male-sterile cytoplasm to permit handling of large numbers of pollinations necessary for success. Initial target genes are Rf4, the restorer gene for cms-C, and Hm1, the gene which confers resistance to Helminthosporium carbonum, Race 1. Two graduate students are using RFLP markers to localize genes for resistance to grey leaf spot disease (Cercospora zeae-maydis) and for

photoperiodism in maize. Important genetic stocks, hm1, ms 14, br2, f, bz2, gs1, and bm2 have been developed and are being sent to the Maize Genetics Stock Center in Urbana, IL, for preservation and disbursement. These stocks provide linked coverage of the whole of the long arm of Chromosome One of maize. More detailed genetic map of Chromosome 1-L is being developed for use in gene localization and tagging.

Publications: 88/01 to 88/12
AGNELL, D.M., SISCO, P.H., and STUBER, C.W.
 1988. Linkage relationships between isozyme
 and morphological markers on chromosome 1L.
 Maize Genetic Coop. News Lett. 62:102.
SISCO, P.H. 1988. Mapping: Chromosome 1-L.
 Maize Genetic Coop. News Lett. 62:124.

14.055 CRISO133773 DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS

STUBER C W; Agricultural Research Service,
Raleigh, NORTH CAROLINA 27607.
Proj. No.: NCR-8702804 Project Type: CRG0
Agency ID: CRG0 Period: 15 SEP 86 to 30 SEP 89

Objectives: Use molecular markers (isozymes and RFLP's) to identify, locate and enumerate quantitative trait loci (QTL's) that contribute to the heterotic response in a cross of two widely-used elite maize inbred lines and evaluate the magnitudes of the effects and types of gene action associated with these individual loci (or small chromosomal segments). Identify and locate genetic factors in two additional inbred lines that will be used to enhance the heterotic response of the original elite hybrid. PROJ. 8702804.

Approach: Development of a set of F3 lines (about 320) from the cross of the elite hybrid. These will be backcrossed to the two parental lines. Backcross progeny will be evaluated in replicated field trials and results will be used to determine the marker-associated effects on heterosis of several traits. For the second objective, a similar approach will be used in that F3 lines will be developed from the cross of two other appropriately chosen inbred lines. These F3 lines will be crossed to the two elite lines used in the original heterotic hybrid. Evaluations of these crosses will be made and comparisons will be made among appropriate genotypes to determine which factors should be transferred to the original elite inbred lines.

14.056 CRISO141000 DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS

STUBER C W; Agricultural Research Service, Raleigh, **NORTH CAROLINA** 27607. Proj. No.: 0101-21020-008-84R

Project Type: INHOUSE Agency ID: ARS Period: O1 OCT 86 to 30 SEP 89

Objectives: Use molecular markers (isozymes and RFLP's) to identify, locate and enum- erate quantitative trait loci (QTL's) that contribute to the heterotic response in a cross of two widely-used elite maize inbred lines and evaluate the magnitudes of the effects and types of gene action associated withthese individual loci (or small chromosomal segments). Identify and locategenetic factors in two additional inbred lines that will be used to enhancethe heterotic response of the original elite hybrid.

Approach: Development of a set of F3 lines (about 320) from the cross of the elite hybrid. These will be backcrossed to the two parental lines. Backcross progeny will be evaluated in replicated field trials and results will be used to determine the marker-associated effects on heterosis of several traits. For the second objective, a similar approach will be used in that F3 lines will be developed from the cross of two other appropriately choseninbred lines. These F3 lines will be crossed to the two elite lines used in the original heterotic hybrid. Evaluations of these crosses will be made and comparisons will be made among appropriate genotypes to determine which factors should be transferred to the original elite inbred lines. FUNDED BY USDA COMPETITIVE GRANTS PROGRAM.

Progress: 88/01 to 88/12. In the previous season, 264 F3 lines from the cross of B73 X MO17 were backcrossed to the parental lines. Also, 216 F3 lines from the cross of Oh43 X Tx303 were testcrossed to B73 and Mo17. The F3 lines were assayed for their isozyme and RFLP genotypes at 77 loci in collaboration with Native Plant Institute. Field evaluations of eight quantitative traits in the backcross and testcross progenies were conducted on 6000 field plots in North Carolina, Iowa, and Illinois. Data analyses indicate that genetic factors affecting the expression of heterosis were distributed throughout the genome for most traits. For grain yield, the factors associated with the greatest effects were concentrated on chromosomes 1 through 5, however. Locations of factors affecting the expression of ear leaf area and plant height were very similar to those found for grain yield. For several quantitative traits, locations of factors affecting their expressions were similar to those found in eight F2 populations studied in earlier experiments. Chromosomal segments were identified in Oh43 and Tx303 that would be expected to enhance the heterotic response of the B73 X Mo17 hybrid. Marker-facilitated backcrossing of factors from Oh43 to Mo17 and from Tx303 to B73 is now being conducted. There was relatively little evidence for interaction of these genetic effects with environments.

Publications: 88/01 to 88/12

STUBER, C.W. and HELENTJARIS, T. 1988.

Molecular markers for dissecting and enhancing heterosis in an elite maize hybrid. Agronomy Abstracts, p. 97.

BRIDGES, W.C., KNAPP, S.J., STUBER, C.W., and EDWARDS, M.D. 1988. Molecular marker-facilitated investigations of quantitative-trait loci in maize. III. Epistasis and alternative model building strategies. Agronomy Abstracts p. 75.

14.057 CRISO141416 GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS

STUBER C W; Agricultural Research Service, Raleigh, NORTH CAROLINA 27607. Proj. No.: 6645-21220-002-00D

Project Type: INHDUSE

Period: 31 MAR 86 to 31 MAR 91 Agency ID: ARS

Objectives: (1) Develop and utilize molecular markers to study the genetic basis of quantitatively inherited traits in corn. including identifying and locatingquantitative trait loci (QTL's), deducing magnitudes and types of gene action, and utilizing markers for manipulating performance of quantitative traits. (2) Develop, evaluate, and utilize breeding systems and recurrent selection techniques for incorporating exotic sources into U.S. corn germplasm pools and for improving these pools.

Approach: Develop appropriate populations, such as F2 populations, that are segregating at numerous marker loci. Characterize individual plants for marker genotypes and evaluate quantitative traits on these plants in field trials. Analyze associations of quantitative measures with marker genotypes to locate QTL's and study magnitudes and types of gene action at these loci. Based on this information, conduct marker-facilitated selection for desired traits. Evaluate effects of specific QTL's using marker-facilitated approaches in various tester backgrounds and in various environments to determine genetic and environmental stability. Use marker-facilitated approaches to identify and transfer desirable alleles from exotic to domestic germplasm. Use sevral selection procedures (mass, full-sib, modified reciprocal full-sib) to improve several traits inpopulations with 25 to 100% exotic germplasm.

Progress: 88/01 to 88/12. The major effort in this project continues to be focused on the use of molecular markers, isozyme loci and RFLPs, to identify and locate quantitative trait loci (QTL) affecting grain yield and several agronomic traits in corn. Analyses of data from field evaluations of 190 S6 lines (from the cross CD159 X Tx303) per se and of testcrosses of these lines to three inbred testers (Dh43, A632, and K55) are nearly completed. The lines were genotyped at about 60 marker loci (isozyme and RFLP), and field data were recorded on 8 quantitative traits. QTL identified in the lines per se appeared to be similar to those identified in previous F2 population studies. Locations of factors associated with the expression of quantitative traits in the testcrosses differed from those found in the lines per se, and there was evidence for considerable interaction of effects with genetic background. There was, however, little evidence for interaction of genetic effects with environment. Selection studies are continuing in a number of widely divergent corn populations with either 100 or 50 percent Latin American germplasm. A number of F1 hybrids were generated from inbred lines developed from a modified reciprocal full-sib selection program in populations with 50

percent exotic germplasm. Field evaluations of these hybrids were made and preliminary analyses indicated that some of these hybrids vielded better than the best commercial checks available and also showed other desireable agronomic traits.

Publications: 88/01 to 88/12

DDEBLEY, J.F., WENDEL, J.F., SMITH, J.S.C., STUBER, C.W., and GODDMAN, M.M. 1988. The origin of cornbelt maize: The isozyme evidence. Economic Botany 42:120-131.

STUBER, C.W., WENDEL, J.F., GDODMAN, M.M., and SMITH, J.S.C. 1988. Techniques and scoring procedures for starch gel eletrophoresis of enzymes from maize (Zea mays L.). NCARS-NCSU Tech. Bull. 286, 87

BURR, R., BURR, F.A., THDMPSDN, K.H., ALBERTSDN, M.C., and STUBER, C.W. 1988. Gene mapping with recombinant inbreds in maize. Genetics 118:519-526.

WENDEL, J.F., GDDDMAN, M.M., STUBER, C.W., and BECKETT, J.B. 1988. New isozyme systems for maize: Acon. hydratase, adeny. kinase, NADH dehydrogenase, and shik. dehydrogenase. Biochem. Genet. 26:421-445.

GUFFY, R.D., STUBER, C.W., and HELENTJARIS, T. 1988. Molecular markers for evaluating quantitative traits across varying genetic backgrounds and environments in maize. Agronomy Abstracts p. 82.

14.058 CRIS0140139 ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI

STUBER C W; Agricultural Research Service; Agricultural Research Service, Raleigh, NORTH CAROLINA 27607.

Proj. No.: 0101-21020-008-03R

Project Type: INHOUSE

Agency ID: ARS Period: 15 SEP 83 to 30 SEP 85

Objectives: 1) To use isozyme loci as markers to locate genes or chromosomal segments that have major effects on the expression of quantitatively inherited traits in maise, and 2) to investigate responses of quantitative traits to selection based solely on the manipulation of allelic frequencies of isozyme loci in composite populations consisting of exotic and U. S. corn germplasm.

Approach: For objective (1), enzyme characteristics and quantitative traits will be measured on individual plants in the F2 of a cross of two inbred lines withmore than 14 segregating isozyme marker loci. Association of quantitative traits with marker loci will be based on results from yield testing a largenumber of full-sib families in the composite population and associations of isozyme alleles with highest and lowest yielding families. Results of isozyme based selections will be yield tested to measure response. This isa Competitive Grants Project.

Progress: 86/01 to 86/12. From studies of F2 populations generated from the crosses, CO159 \times T \times 303 and T232 \times CM37, it was concluded that segregating isozyme loci in F2 populations

provide an effective means for identifying chromosomal regions that affect a wide array of phenotypic characteristics in corn. For the 82 quantitative traits evaluated, differences in trait expression were found to be significantly associated with marker -locus genotypes in 830 of 1394 comparisons (60%) in the first population and in 1079 of 1640 comparisons (66%) in the second population. Factors contributing as little as 0.2% of the phenotypic variation in quantitative traits were detected, with some individual marker loci explaining as much as 17% of the phenotypic variation. Studies in six F2 populations generated from crosses of B73, Mo17, and Oh43 to Tx303 and T232 produced similar results. Thus, even in crosses among lines that are similar morphologically, isozyme marker loci were effective in identifying quantitative trait loci. Isozyme loci recently mapped include Acp4, Dia2, Adk1, Tpi3, and Sad1.

Publications: 86/01 to 86/12

WENDEL, J.F., GOODMAN, M.M. and STUBER, C.W. 1986. Additional mapping of isozyme loci: Localization of Acp4, Dia2, Adk1, Tpi3, and Sad1. Maize Genet. Coop. New Lett. 60:109-110.

BATEMAN, B.S., STUBER, C.W., EDWARDS, M.D. and WENDEL, J.F. 1986. Use of allozme markers for identifying quantitative trait loci in crosses of elite lines in maize. Agronomy Abstracts, p. 56.

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WENDEL, J.F., STUBER, C.W., EDWARDS, M.D. and
GOODMAN, M.M. 1986. Duplicated chromosome
segments in Zea mays L.: Further evidence
from hexokinase enzymes. Theor. Appl.
Genet. 72:178-185.

14.059 CRISO097934 ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION AND FUNCTION

SISCO P H; Crop Science; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO5614 Project Type: STATE Agency ID: SAES Period: O1 OCT 85 to 30 SEP 90

Objectives: Isolate and characterize corn genes of potential use for crop improvement. Create isolines for disease-resistance and susceptibility. Investigate double-stranded RNA molecule associated with cytoplasmic male sterility.

Approach: Transposable elements will be used to tag and isolate genes of interest and to create isolines. Nucleic acid and protein sequence of genes will be determined. A cDNA copy of double-stranded RNA molecule for sequencing will be made. Expression vectors will be used to produce the protein encoded by the genes, so that gene function can be investigated.

Progress: 88/01 to 88/12. Work is progressing to isolate agronomically important genes from corn using the transposable elements Activator, Suppressor Mutator, and Robertson's Mutator as tags. A large isolation block will be grown out in the 1989 nursery. Restriction fragment length polymorphism (RFLP) markers are

being used to improve the B73 x Mo17 heterotic response by incorporating selected chromosome segments from Tx303 and Oh43, respectively. RFLP's are also being used to identify chromosome segments responsible for photoperiodism in tropical maize and gray leaf spot resistance from several maize sources.

Publications: 88/01 to 88/12
SISCO, P.H. 1988. Mapping Chromosome 1-L.
Maize Genet. Coop. News Lett. 62:124.
AGNELL, D.M., SISCO, P.H., and STUBER, C.W.
1988. Linkage relationship between isozyme and morphological markers on chromosome 1L.
Maize Genet. Coop. News Lett. 62:102.

14.060 CRISOO66377 MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES

GOODMAN M M; STUBER C W; Genetics; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO5304 Project Type: STATE Agency ID: SAES Period: 01 OCT 84 to 30 SEP 89

Objectives: Measure divergence of races of maize and teosinte based on isozyme frequencies in studying their evolution, migration, and degrees of diversity; develop systems of classification for within-species variation; investigate linkage disequilibria in these populations to check conclusion reached on the basis of less comprehensive collections; study the restriction of recombination in exotic by adapted crosses to enable breeders to optimize breeding procedures in such crosses; develop marker stocks for use in quantifying the degree of cryptic cytological differentiation within maize and teosinte.

Approach: We plan to score the electrophoretic genotypes of 12 to 24 plants of about 1000 typical collections of the 250 or so races of maize for about 20 isozyme loci. We will use distance measures and cluster analysis to group the races, hopefully improving the groupings based on previously published data. Extreme variants will be combined to provide multiple marker stocks for use in assaying recombination frequency differences and selected maize and teosinte collections.

Progress: 87/01 to 87/12. Analyses of isozyme allelic frequency data generated from more than 1000 collections of Latin American races of maize is continuing using numerical taxonomic procedures. An examination of isozyme variation in 67 accessions representing the indigenous Guatemalan maize races revealed that highland races were isozymatically distinct from lowland races. The race Nal-Tel was shown to contain at least 2 isozymatically and ecogenetically distinct forms that might be considered distinct races. As in the previous analyses of Bolivian and Mexican races, occurrence of certain isozyme alleles was strongly associated with altitude. Isozyme assays have recently been completed on additional collections from Mexico, southwestern and northern United States to fill in gaps in the surveys of maize variation from

North and South America. Results from these assays will also be analyzed using numerical taxonomic procedures.

Publications: 87/01 to 87/12

DOEBLEY, J. F., M. M. GOODMAN, and C. W. STUBER. 1987. Patterns of isozyme variation between maize and Mexican annual teosinte. Economic Botany 41:234-246.

WENDEL, J. F., M. D. EDWARDS, and C. W. STUBER. 1987. Evidence for multilocus genetic control of preferential fertilization in maize. Heredity 58:297-301.

BRETTING, P. K., M. M. GOODMAN, and C. W. STUBER. 1987. Karyological and isozyme variation in West Indian and allied American mainland races of maize. Am. J. Botany 74:1601- 1613.

SISCO, P. H., J. F. WENDEL, and C. W. STUBER. 1987. Acp4 is the most distal marker on chromosome 1L. Maize Genetic Coop Newsletter 61:86.

14.061 CRISO094151 DEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.

LEVINGS C S; Genetics; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO3911 Project Type: HATCH Agency ID: CSRS Period: 01 NOV 84 to 30 SEP 89

Objectives: To identify, isolate and characterize mitochondrial genes of corn and tobacco. To develop a transformation system for mitochondrial genes.

Approach: We will identify and characterize the protein genes of the maize and tobacco mitochondrial genomes. Mitochondrial genes are known to code for important gene products crucial for respiration and oxidative phosphorylation, and for the trait cytoplasmic male sterility which is useful in hybrid seed production. These studies will provide information concerning the coding capacity of plant mitochondrial genomes as well as identify possible genes for future genetic engineering studies. In addition, we will carry out experiments aimed at developing a transformation system for mitochondrial genes.

Progress: 88/01 to 88/12. The urf13 gene of cms-T mitochondrial encodes a 13 kD polypeptide that specifically interacts with the pathotoxin, T-toxin, produced by Bipolaris maydis, race T. This interaction causes massive ion leakage, swelling, and the inhibition of respiration in maize mitochondria and E. coli cells expressing the 13 kD protein. We have carried out site-directed mutagenesis of the urf13 gene to determine the amino acid residues that are important in conferring toxin sensitivity to E. coli. These analyses have identified mutant URF13 polypeptides that are no longer able to confer toxin sensitivity. Thus it may be possible to alter the urf13 gene so that it does not confer sensitivity to toxin and susceptibility to the fungal disease, Bipolaris maydis, race T. The urf13 gene is also implicated in causing cytoplasmic male

sterility in cms-T maize. We are investigating whether the 13 kD polypeptide can be altered to eliminate toxin sensitivity and still retain its ability to cause cytoplasmic male sterility. We are attempting to identify the maize mitochondrial genes responsible for the C type (cms-C) of cytoplasmic male sterility. Three mutant genes have been found in cms-C that are correlated with sterility; they include mutant forms of the atp9, atp6 and coxII genes. All of these mutant genes have arisen by rearrangements that have altered their promoter sites, and in the case of coxII and atp6, their coding regions. These studies indicate the importance of rearrangements in causing plant mitochondrial gene mutations.

Publications: 88/01 to 88/12

BLAND, M. M., C. S. LEVINGS III, and MATZINGER, D. F. 1987. The ATPase subunit 6 gene of tobacco mitochondria contains an unusual sequence. Curr. Genet. 12:475-481.

GWYNN, B., R. E. DEWEY, R. R. SEDEROFF, D. H. TIMOTHY and C. S. LEVINGS III. 1987. Sequence of the 185-55 ribosomal gene region and the cytochrome oxidase II gene from mtDNA of Zea diploperennis. Theor. Appl. Genet. 74:781-788.

DEWEY, R. E., J. N. SIEDOW, D. H. TIMOTHY and C. S. LEVINGS III. 1988. A 13 kD maize mitochondrial protein in E. coli confers sensitivity to Bipolaris maydis toxin. Science 239:293-295.

LEVINGS, C. S. III and R. E. DEWEY. 1988.
Molecular studies of cytoplasmic male
sterility in maize. Phil. Trans. R. Soc.
Lond. B 319:177-185.

14.062 CRISO095811 SYSTEMS FOR THE MOLECULAR ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES

SCANDALIOS J G; Genetics; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695. Proj. No.: NCO3927 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 90

Objectives: To utilize a number of gene-enzyme systems we have developed as "models" to study the genetic and molecular mechanisms by which higher eukaryotic organisms regulate the differential expression of specific genes during development and differentiation.

Approach: Specific gene-enzyme systems defined by us genetically and biochemically, will be used as probes to study the underlying molecular mechanisms regulating differential gene expression during maize development. The response of specific genes to both genetic and environmental signals will be studied. Experimental approaches will involve such current molecular techniques as gene cloning, sequencing and in vitro translation assays, as well as conventional molecular and genetic techniques.

Progress: 88/01 to 88/12. During the past year we utilized the Sod and Cat genes which we isolated, cloned, sequenced and characterized to further investigate the molecular bases for:

(a) the differential expression of genes during development; (b) the response of antioxidant genes to environmental stress; and (c) the molecular bases for the intracellular protein trafficking of the protein products of these genes. Specifically, we demonstrated that the steady-state level of the Cat3 transcript varies with the time of day the plants are sampled. This diurnal variation is due to a circadian rhythm likely to result from differential transcription of the Cat3 gene. Also, removal of the embryonic axis prior to imbibition causes the level of mRNA encoding glyoxysomal proteins to drop sharply. This suggests that a signal from the axis regulates the level of expression of these genes (e.g., Cat2). Through in vitro mutagenesis we identified regions of the SOD-3 transit peptide necessary for the efficient import of \$0D-3 into the mitochondria: this being the first such analysis of a mitochondrial precursor protein in plants. Tobacco leaf tissue transfected with Sod/Gus promoter fusions using a particle accelerator ("gene gun") showed that the maize Sod4 promoter is active in tobacco. We will use transgenic plants to examine the stress response of these genes. Additionally, we developed a procedure for the small-scale isolation of plant RNA, essential for our studies of gene expression in developing tissues.

Publications: 88/01 to 88/12

BETHARDS, L. A. and SCANDALIOS, J. G. 1988.
Molecular basis for the CAT-2 null
phenotype in maize. Genetics 118:149-153.

WADSWORTH, G. J., REDINBAUGH, M. G. and SCANDALIOS, J. G. 1988. A procedure for the small-scale isolation of plant RNA suitable for RNA blot analysis. Analyt. Biochem. 172: 279-283.

SCANDALIOS, J. G. 1988. Genomic responses to environmental stress: the antioxidant genes of maize. In: "The Genetics and Evolution of Eukaryotes." USSR Acad. Sci. Press, Moscow, pp. 1-12.

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Molecular biology of intracellular protein trafficking. Physiol. Plant. 74:397-408.

WHITE, J. A. and SCANDALIOS, J. G. 1988.
Isolation and characterization of a cDNA
for mitochondrial Mn-SOD-3 of maize and its
relation to other Mn-SODs. Biochim.
Biophys. Acta 951:61-70.

REDINBAUGH, M. G., WADSWORTH, G. J. and SCANDALIOS, J. G. 1988. Characterization of catalase transcripts and their differential expression in maize. Biochim. Biophys. Acta 951:104-116.

14.063 CRISOO34140 ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI

STUBER C W; Genetics; North Carolina State University, Raleigh, NORTH CAROLINA 27695. Proj. No.: NC-8300491 Project Type: CRGD Agency ID: CRGO Period: 15 SEP 83 to 30 SEP 86

Objectives: Project 8300491. To use isozyme loci as markers to locate genes or chromosomal segments that have major effects on the

expression of quantitatively inherited traits in maize, and to investigate responses of quantitative traits to selection based solely on the mainipulation of a allelic frequencies of isozyme loci in composite populations consisting of exotic and U.S. corn germplasm.

Approach: For objective (1), enzyme characteristics and quantitative traits will be measured on individual plants in the F2 of a cross of two inbred lines with more than 14 segregating isozyme marker loci. Association of quantitative traits with marker will be tested statistically. For objective (2) manipulation of allelic frequencies of iszyme) loci will be based on results from yield testing a large number of full-sib families in the composite population and associations of isozyme alleles with highest and lowest yielding families. Results of isozyme based selections will be yield tested to measue response.

14.064 CRISO091503 RECOMBINANT DNA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY

OLESON A E; BERRYHILL D L; KOFOID K D; Biochemistry; North Dakota State University, Fargo, NORTH DAKOTA 58105. Proj. No.: NDO1228 Project Type: HATCH Agency ID: CSRS Period: O1 NOV 83 to 30 SEP 88

Objectives: Clone stress response genes from selected plant species and determine the nucleotide sequences of their polypeptide reading frames and flanking regulatory regions. Elucidate the dynamics of expression of stress response genes under various stress conditions by use of recombinant DNA probes. Clone and determine the sequence of nuclear genes for ribosomal RNA from higher plants. Assess mitochondrial diversity in wheat with recombinant DNA techniques. Determine the effects on wheat mitochondrial DNA of growth in tissue culture and regeneration.

Approach: A generalized stress-response plant enzyme (RNase I) will be purified, partially sequenced (Edman method), and a synthetic oligonucleotide probe prepared. Clones from cDNA and genomic libraries will be isolated, sequenced, and used as probes of specific mRNA levels in stressed plants. A previously cloned maize rRNA gene in a lambda vector will be subcloned into plasmid and M13 phage vectors, and sequenced by the dideoxy method. Mitochondrial DNA will be isolated from several species of Triticum and Aegilops and restriction maps prepared. Sequence homologies will be determined by blotting procedures. Restriction maps of mitochondrial DNA from a single species subjected to tissue culture and regeneration will also be compared.

Progress: 83/11 to 88/09. Mitochondrial DNA has been isolated from five wheat cultivars. Each has an AABBDD nucleus, but the cytoplasmic genomes came from Aegilops squarrosa, Haynaldia villosa, T. aestivum, T. timopheevi, and T. turgidum. The DNA preparations were examined

with restriction endonucleases BamHI, EcoRI, HindIII, and XhoI. Labeled probes used for this analysis were from T. aestivum or sorghum mitochondrial DNA. The results indicated that Haynaldia villosa was the B genome donor to T. turgidum and T. aestivum. In other work, a lambda clone of the nuclear ribosomal gene region of maize has been subcloned into Escherichia coli plasmid vectors. Shotgun fragments were cloned into a phage vector, and these were sequenced by the dideoxy method. This gene family is present as tandem repeats on the chromosome. The transcript encodes, in a 5' to 3' direction, 17S, 5.8S, and 26S ribosomal RNAs, with internal transcribed spacers 1 and 2 flanking the 5.8\$ region. Clones containing the 5.85 and spacer regions were used as probes for RFLP analysis of corn DNA. These studies indicated that little variation exists in the internal spacer regions of the multiple copies of this gene family. Sequencing of the 26S region was completed. Comparison of the alpha-sarcin domain of this RNA indicated that the 14-base core was the same as that of all other eukaryotes tested. The base flanking the 5'-end of the core is C in the case of all animals tested, whereas U is present at this position in corn, yeast and rice.

Publications: 83/11 to 88/09
MESSING, J.; CARLSON, J.; HAGEN, G.;
RUBENSTEIN, I.; and OLESON, A. 1984.
Cloning and sequencing of the ribosomal RNA
genes in maize: The 17S region. DNA
3:31-40.

14.065 CRISO099204
GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES
ON TWO HOMEOTIC MUTANTS OF MAIZE

SHERIDAN W F; Biology; University of North Dakota, Grand Forks, NORTH DAKOTA 58201.

Proj. No.: NDR-8601841 Project Type: CRGD Agency ID: CRGD Period: O1 JUL 86 to 30 JUN 89

Objectives: The research objectives of the project are to examine: 2) how the two maize mutants, tru and tb. influence the development of axillary meristems and branches. b) the pattern of inheritance, chromosome arm location and linkage map position of tru. c) the interaction of tru and tb with each other mutants and their cellular autonomy. d) the morphology of the meristems and branches of both mutants.

Approach: Genetic characteristics will be studied by making crosses with linkage marker stocks and B-A translocations. Cellular autonomy of mutant expression will be examined by cell clonal analysis using anthocyanin markers. Morphogenesis will be studied by dissection and paraffin sectioning techniques.

Progress: 87/01 to 87/12. During the past year good progress was made on this project. Initial efforts to determine the chromosome arm location of the tru mutant were not completely clear so the T-waxy translocations were used and by fall 1987 tru was located to chromosome 3, probably 3L. In addition both tru and tb

have been crossed into colored plant stocks in preparation for experiments on the clonal analysis of autonomy of mutant expression. Also crosses were made in the winter of 1987 using tru and tb plants as male parents to cross onto active mutator stocks in pursuit of transposon-tagged mutants at these two loci. Work on the detailed mapping of both genes is in progress. Crosses with TB-1La onto +/tb ears and with TB-3La on tru/tru ears were made during winter 1987 in preparation for examining degree of mutant expression in hypoploid plants versus that in diploid plants. Crosses of tru with a day-length sensitive stock have been made with the goal of examining the influence of node number on mutant expression.

Publications: 87/01 to 87/12 NO PUBLICATIONS REPORTED THIS PERIOD.

14.066 CRISO141623 BIOLOGY, EPIDEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN

GINGERY R E; LOUIE R; MCMULLEN M; Agricultural Research Service, Wooster, **OHIO** 44691.

Proj. No.: 3607-22240-001-000

Project Type: INHOUSE

Agency ID: ARS Period: 01 APR 86 to 31 MAR 91

Objectives: a) Devise maize virus and mycoplasma assays, b) elucidate geographical and biological origins of maize mosaic virus, c) characterize maize viruses, d) establish maize chlorotic dwarf etiology, e) isolate vectors of maize white line and maize subtle mosaic viruses, f) assess yield loss caused by maize viruses, g) elucidate virus disease epidemiology.

Approach: a) Monoclonal antibodies against maize viruses will be used to devise assays, b) world-wide MMV isolates will be compared by a variety of tech- niques and analyzed by cladistic methods to determine phylogeny, c) viruseswill be chemically, physically and serologically characterized to determine their impact on corn production, d tissue will be examined for auxiliary particles by double-stranded RNA analyses and particle isolations, e) sus- pected vectors will be isolated by population dilution and selective growing conditions, f) losses due to virus diseases in widely-used inbreds and hybrids will be assessed, g) disease incidence will be correlated with environment, vector populations, and virus sources.

Progress: 88/01 to 88/12. The chromosomal map position of the major gene for resistance to maize dwarf mosaic virus (MDMV) in the inbred PA405 was determined using restriction fragment length polymorphism analysis. The effect on MDMV-induced symptom expression of transferring this gene into two susceptible backgrounds was also determined. Antibodies specific to each of the three maize chlorotic dwarf virus capsid proteins were used to screen gt10 cDNA expression libraries and putative cDNA clones for each of the capsid proteins were isolated. Maize necrotic lesion virus was purified, and the particles had a similar

morphology in EM to those from leaf dip preparations. Maize white line mosaic virus from infected root inocula was consistently transmitted to healthy maize seedlings growing in modified hydroponic conditions. Transmission was unaffected by the fungicides and antibiotics tested. The genes for the capsid and noncapsid proteins of maize strip virus (MStV) were cloned and used to detect the virus in infected plants and insects. A cDNA library to MStV was created and 30 clones of parts of the MStV genome isolated. A new virus, maize yellow stripe virus, was isolated and characterized.

Publications: 88/01 to 88/12

MCMULLEN, M.D., and LOUIE, R. 1988.
Restriction fragment length polymorphism analysis of resistance to maize dwarf mosaic virus. Genome 30, Suppl. 1: 451 (Abstr.).

MILLER, J., OGDEN, S., MCMULLEN, M., HERBERT, A., and STORB, U. 1988. The order and orientation of mouse lambda-rearrangement patterns. J. Immun. 141:2497-2502.

PHILLIPS, R.L., MCMULLEN, M.D., ENOMOTO, S., and RUBENSTEIN, I. 1988. Ribosomal DNA in majze. Stadler Symp. 19:201-214.

maize. Stadler Symp. 19:201-214.

AMMAR, E.D., and LOUIE, R. 1988. Viruslike particles in maize white line mosaic virus-infected roots and in an associated fungus. 5th Int. Congress of Plant Pathology, Kyoto, Japan. Aug. 20-27, 1988. p. 58. (Abstr.).

AMMAR, E.D., GINGERY, R.E., and NAULT, L.R. 1987. Interactions between maize mosaic and maize stripe viruses in their insect vector, Peregrinus maidis, and in maize, Phytopathology 77:1051-1056.

HUNT, R.E., NAULT, L.R., and GINGERY, R.E. 1988. Evidence of infectivity of maize chlorotic dwarf virus and for a helper component in its leafhopper transmission. Phytopathology 78:499-504.

GINGERY, R.E. 1988. The rice stripe virus group. Chapter 9 (pp. 297-329) in: "The Filamentous Plant Viruses" (R.G. Milne, ed.) Vol. 4 in The Plant Viruses (H. Fraenkel-Conrad and R.R. Wagner, eds.) Plenum Press, New York.

14.067 CRISO085330 MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN

TUCHEN-PEI D; Biochemistry & Microbiology; Pennsylvania State University, University Park, **PENNSYLVANIA** 16802.

Proj. No.: PENO2630 Project Type: HATCH Agency ID: CSRS Period: O1 JUL 82 to 30 JUN 86

Objectives: Isolate and purify glutathione s-transerase mRNA sequences; study genome organization and structure of glutatione s-transferases of corn and possibly other plants; investigate molecular basis of herbicide resistance in corn and possible other plants.

Approach: Make partial DNA sequences analysis of selected genomic fragments and select forcomplementary mRNA sequences. Tryptic

mapping of purified corn glutathione s-transferase followed by partial N-terminal analysis of selected fragments. Further characterization of the various cDNA and genomic clones of corn glutathione s-transferases.

Progress: 82/07 to 86/06. We have purified at least two corn GSTs that have activity against CDNB, very low activity against atrazine, from two strains of corn, GT112RfRf and its atrazine-sensitive segregant GTR112. The first activity that elutes from the Mono Q anion exchange column shows a single band on SDS-PAGE at Mr=28K. Later fractions contain two bands, 28K and 27K, indicating a possible heterodimer. The western blots show that the antibody against the 28K subunit has little if any cross reactivity to the 27K subunit(s). The 28K antibody does, however, react with the heterodimer's larger subunit (Mr=28K). This indicates that the larger subunit of the heterodimer may belong to the same family as the homodimer isozyne(s). Using antibody against either isozyme fraction(s) in screening at a lambda gt11 cDNA library constructed from poly(A) RNAs of Gt112 RfRf leaves, we have obtained a total of 18 cDNA clones. Half of the clones selected by the heterodimer antibody did not hybridize to cDNAs selected by the homodimer antibody, thus suggesting the presence of at least two separate genes (or gene families) for the corn GSTs. We have also purified the major atrazine-conjugation GST from GT112RfRf to electrophoretic homogeneity. The pronounced differences in activity and antigenicity amoung the corn GSTs may be evidence for a corn GST supergene family, similar to that found in rats.

Publications: 82/07 to 86/06
NO PUBLICATIONS REPORTED THIS PERIOD.

14.068 CRISO034080 THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN AND COTTON

TU C P D; GROVE G; LI N; Biochemistry Microbiology Molecular & Cell Biology; Pennsylvania State University, University Park, PENNSYLVANIA 16802.

Proj. No.: 8300456 Project Type: CRGD Agency ID: CRGO Period: 01 SEP 83 to 31 AUG 86

Objectives: Project Number 8300456. We inend to study the molecular genetics of the glutathione S-transferase (GST) genes from corn and cotton. We will isolate the GST isozymes from corn, prepare antisera against corn GSTs, construct cDNA plasmids for corn GST mRNA sequences and isolate genomic sequences from constructed gene libraries of corn and cotton. Our long-term goal is to establish the relationship between GST isozymes expression and selected herbicide resistance (e.g. atrazine resistance) in corn. The GST gene sequences can be manipulated in vitro for their expression in heterospecific environments.

Approach: The GST isozymes will be purified by a procedure involving S-hexylglutathione linked sephanose 6B affinity column chromatography.

The GST cDNA plasmids will be characterized by hybrid-selected in vitro translation and DNA sequence analysis. Genomic sequences will be isolated from gene libraries with specific cDNA probes. The GST isozyme(s) involved with atrazine resistance will be identified and characterized from an atrazine sensitive corn line.

Progress: 86/09 to 87/11. We have isolated from a constructed lambda qtll expression library two classes of cDNA clones encoding the entire sequence of the maize GSH S-transferases GST I and GST III. Expression of a full-length GST I cDNA in E. coli resulted in the synthesis of enzymatically active maize GST I that is immunologicaly indistinguishable from the native GST I. Another GST I cDNA with a truncated N-terminal sequence is also active in heterospecific expression. Our GST III cDNA sequence differs from the version reported by Moore et. al (Moore, R. E., Davies, M. S., O'Connell, K. M., Harding, E. I., Wiegand, R. C., and Tiemeier, D. C. (1986) Nucleic Acids Res. 14:7227-7235) in eight reading frame shifts which result in partial amino acid sequence conservation with the rat GSH S-transferase sequences. The GST I and GST III sequences share cycle sine 45% amino acid sequence homology. Both the GST I and the GST III mRNAs contain different repeating motifs in front of the initiation codon ATG. Multiple poly(A) addition sites have been identified for these two classes of maize GSH S-transferases messages. Genomic Southern blotting results suggest that both GST I and GST III are present in single or low copies in the maize (GT112 RfRf) genome. We have achieved the original objectives of the proposal: to purify GST isozymes from corn, to prepare antisera against these purified maize GST isozymes, to construct cDNA libraries (lambda gt11 vector and plasmid vectors), to isolate and characterize GST cDNAs, to express corn cDNAs in E.

Publications: 86/09 to 87/11

- TIMMERMAN, K.P. and TU, C.-P.D. 1987. Genetic evidence of xenobiotics metabolism by GSH S-transferases from corn in "Glutathione S-Transferases and Carcinogenesis" pp 47-49, Edited by T.J. Mantle, C.B. Pickett and J.D. Hayes.
- TIMMERMAN, K.P. 1987. Purification and characterization of corn glutathione S-Transferases, Ph.D. Thesis. The Pennsylvania State University, University Park, PA. 93 p.
- GROVE, G., ZARLENGO, R.P., TIMMERMAN, K.P., LI, N., TAM, M.F. and TU, C.-P.D. 1988. Characterization and heterospecific expression of cDNA clones of genes in the maize GSH S-transferase multigene family. Nucleic Acids Res. 16:(in press).

14.069 CRISO130014 MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN

TU C P D; QIAN B; GROVE G; Molecular and Cell Biology; Pennsylvania State University, University Park, **PENNSYLVANIA** 16802.
Proj. No.: PENR-8601652 Project Type: CRG0

Agency ID: CRGO Period: O1 SEP 86 to 30 JUN 89

Objectives: PROJECT 8601652. We intend to establish the molecular basis of GSH S-Transferase mediated atrazine tolerance in corn. The two atrazine conjugation GST isozymes from corn will be purified to homogeneity, and their kinetic parameters and partial amino acid sequences will be determined. The corresponding CDNA and genomic clones will be identified and characterized in order to establish the "mutation" in the atrazine sensitive corn line GT112.

Approach: The atrazine specific corn GST isozymes will be further purified by a combination of hydrophobic interaction, gel filtration, and ion exchange chromatography. Partial amino acid sequences will be determined from these GST isozymes and will aid in the identification of the corresponding GST cDNA clones. The molecular basis of the "mutation" in GT112 will be determined by mapping and sequencing cDNA and genomic clones corresponding to the atrazine specific corn GST isozymes.

Progress: 86/09 to 87/08. We have isolated from a constructed gamma gt11 expression library two classes of cDNA clones encoding the entire sequence of the maize GSH S-transferases GST I and GST III. Expression of a full-length GST I cDNA in E. coli resulted in the synthesis of enzymatically active maize GST I that is immunologically indistinguishable from the native GST I. Another GST I cDNA with a truncated N-terminal sequence is also active in heterospecific expression. Our GST III cDNA sequence differs from the version reported by Moore et. al. (Moore, R.E., Davies, M.S., O'Connell, K.M., Harding, E.I., Wiegand, R.C., and Tiemeier, D.C. (1986) Nucleic Acids Res. 14:7227-7235) in eight reading frame shifts which result in partial amino acid sequence conservation with the rat GSH S-transferase sequences. The GST I and GST III sequences share difference 45% amino acid sequence homology. Both the GST I and the GST III mRNAs contain different repeating motifs in front of the initiation codon ATG. Multiple poly(A) addition sites have been identified for these two classes of maize GSH S-transferases messages. Genomic Southern blotting results suggest that both GST I and GST III are present in single or low copies in the maize (GT112 RfRf) genome. In addition, we have purified one putative atrazine-conjugating GST to homogeneity and prepared antiserum against thi protein composed of 25.5 kd subunit. We have also made a lambda gt11 cDNA library from leaf poly(A) RNAs of GT112 (an atrazine sensitive corn line). cDNA screenig has been underway.

Publications: 86/09 to 87/08

- TIMMERMAN, K.P. and TU, C.-P.D. 1987. Genetic evidence of xenobiotics metabolism by GSH S-transferases from corn in "Glutathione S-Transferases and Carcinogenesis" pp 47-49, Edited by T.J. Mantle, C.B. Pickett and J.D. Hayes.
- TIMMERMAN, K.P. 1987. Purification and characterization of corn glutathione S-Transferases, Ph.D. Thesis. The Pennsylvania State University, University

Park, PA. 93 p.
GROVE, G., ZARLENGO, R.P., TIMMERMAN, K.P.,
LI, N., TAM, M.F. and TU, C.-P.D. 1988.
Characterization and heterospecific
expression of cDNA clones of genes in the
maize GSH S-transferase multigene family.
Nucleic Acids Res. 16:(in press).

14.070 CRISO093318 DNA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEDLING

KIM B D; Plant Science; University of Rhode Island, Kingston, **RHODE ISLAND** 02881.

Proj. No.: RI00539 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 87

Objectives: Study control of gene expression at the level of DNA methylation by comparing specific gene sequences and their products between shoot and root organs of maize seedlings.

Approach: Levels of methylation of nuclear and mitochondrial DNAs from shoot and root will be determined by HPLC. Relationships of DNA methylation and the organ-specific gene expression will be probed by utilizing plasmid-like DNAs and other organ-specific cDNAs in combination with methylation-sensitive restriction endonuclease digestion. DNA methyltransferase will be isolated. Organ-specifically expressed DNA sequence(s) will be identified by utilizing cDNAs and recombinant DNAs that will be constructed from organ specific mRNAs. Shoot- and root-specific proteins will be identified by two-dimensional gel electrophoresis using 4-day old, etiolated maize seedlings.

Progress: 84/10 to 87/09. Presence of 5-methylcytosine (5mC) in maize mtDNA and ncDNA was detected by HPLC. The ratio of methylation, 5mC/(C+5mC), was 8.6% and 14.7% for shoot and root mtDNA, respectively; 22.2% and 29.7% for shoot and root ncDNA, respectively. Both mtDNAs from shoot and root of 4-day old maize seedlings were compared by restriction endonuclease digestion patterns. No differences between shoot and root mtDNAs were detected when digested with Eco RI, Hin dIII, Bam HI and Xho I. Two pairs of methylation-sensitive isoschizomers were also tested: no differences between shoot and root mtDNAs were detected when they were digested with Msp I and Hpa II pair and Bst NI and Eco RII pair. Reasons of the possible descrepancy in results of HPLC and restriction endonuclease digestion on the methylation of maize mtDNA are not understood. In vitro tissue culture conditions were found for inducing differential development of maize root and shoot after the initial induction of callus or "embryo-like" structures. Calli were produced from 2mm thick coleoptile segments on 10 uM picloram plates. After transfer to a HF solid media and calli produced green growth centers (GGC) the subcultures of which subsequently regenerated into whole plants. Thus we have developed from a rather recalcitrant coleoptile segments a tissue culture method to either regenerate whole maize plant or produce roots only. This method will

be further developed as a tool of studying differentiation of shoot and root systems in majze.

Publications: 84/10 to 87/09
MAJZA, S.J. 1986. Picloram induced plant
regeneration from Zea mays shoot tissue.
M.S. Thesis. University of Rhode Island,
Kingston. 78 pp.

14.071 CRISO137809 CELLULAR AND MOLECULAR GENETICS FOR IMPROVEMENT OF MAIZE AND FESCUE

FERGUSON N H; Agronomy & Soils; Clemson University, Clemson, **SOUTH CAROLINA** 29634. Proj. No.: SCO1313 Project Type: HATCH Agency ID: CSRS Period: O5 APR 89 to 30 DEC 92

Objectives: To identify strains of the tall fescue endophyte Acremonium coenophialum and to correlate molecular genetic markers with observed differences in plant response. To analyze development of male and female flowers in maize.

Approach: Strains of endophyte will be identified using RFLP analysis. RFLP markers will be correlated with differences in plant response. Interaction of grass and endophyte will be examined using molecular genetic analysis. Flower development in maize will be manipulated using tissue culture technology. Molecular genetic analysis will help identify differences in genes and gene expression.

14.072 CRISO099653 STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE

HUANG A; Biology; University of South Carolina, Columbia, **SOUTH CAROLINA** 29208.

Proj. No.: SCR-8601668 Project Type: CRGO Agency ID: CRGO Period: 15 AUG 86 to 30 AUG 88

Objectives: PROJ 8601668. The project is a study of the structure of genes involved in oil biosynthesis, using maize as a model system. Since there is no existing information (in a plant or any other system) upon which to build, the project is designed to provide a basic foundation for future studies of modifying oil quantity and quality.

Approach: For simplicity in study and importance in triacylglycerol synthesis, genes of five proteins are targeted. Of the five proteins, three are closely related "structural" proteins and the fourth is an amphiphilic "structural" protein of the lipid bodies. The fifth protein is an endoplasmic reticulum enzyme, diacylglycerol acyltransferase, which is the only enzyme known unique to triacylglycerol biosynthesis in the acylglycerol metabolic pathway. These proteins will be purified and partially characterized, and their mode of biogenesis pinpointed. Their genes will be studied by selecting and

sequencing the cONA and genomic clones, analyzing the nucleotide sequences and their deduced amino acid sequences, and probing the mode of their temporal and tissue-specific expression.

14.073 CRISO034107 GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPODIUM)

WILSON H O; Biology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX-8300570 Project Type: CRGO Agency IO: CRGO Period: 15 SEP 83 to 30 SEP 87

Objectives: Project 8300570. Develop information that will provide a better understanding of isozyme analysis as an analytical tool for various techniques associated with genetic engineering, germplasm acquisition, and seed-stock production. Expand the genetic database for important domesticates in the genera Cucurbita and Chenopodiu. Examine the relationship between isozyme variation and genome duplication, domestication, and morphological variation.

Approach: Screen accessions of domesticated and wild species of Cucurbita and Chenopodium for electrophoretic variation. Establish genetic identity for all electrophoretic variants observed. Contrast patterns of genetic variation against selective status (domesticated vs. wild), taxonomic position, geographic distribution, and level of ploidy. Examine F2 families for possible linkage between isozyme loci and morphological characteristics.

Progress: 83/09 to 87/09. Electrophoretic analysis of Cucurbita populations reveals extensive gene duplication resulting from allotetraploidly and unusually high levels of allozyme polymorphism. The pattern of electrophoretic variation places cultivars of C. pepo into two, well defined groups. The "ovifera" group (gourds, crooknecks, pattypans, acorn squash) is aligned with one wild type (C. texana), whereas the "pumpkin" group (pumpkins, marrows, and Mexican landraces) is aligned with another (C. fraterna). Electrophoretic diversity in this primary crop/weed gene pool is centered in commercial cultivars of the "ovifera" group. Native Mexican landraces and wild types are relatively uniform. Interspecific (C. pepo/C. texana) gene flow among experimental populations separated by over 1300 m was documented. Analysis of an F(2) family (C. pepo \times C. texana) suggests a multigenic basis for morphological differentiation between weed and crop with no evidence strong linkage among either morphological or electrophoretic characters. In contrast to out-crossing squash, cultivars of the South American grain chenopod (Chenopodium quinoa), primarily autogamous, show minimal levels of electrophoretic variation. The pattern of variation in both allozymes and morphometric data reflects two groups, Andean and coastal. Morphogenetic differentiation that

exists between Andean and coastal cultivars does not exist between Andean cultivars and free-living (weed, wild) populations of the Andes.

Publications: 83/09 to 87/09

- KIRKPATRICK, K.J. 1984. The Relationship between isozyme phenotype and morphological variation in Cucurbita. M.S. Thesis, Biology, Texas A&M.
- OECKER, O.S. 1985. A biosystematic study of Cucurbita pepo. Ph.O. Thesis, Biology, Texas A&M.
- WALTERS, T.W. 1985. Analysis of systematic and phyletic relationships among alveolate-fruited Chenopodium of western North America. Ph.O. Thesis, Biology, Texas A&M.
- KIRKPATRICK, K.J., OECKER, O.S. and WILSON, H.O. 1985. Allozyme differentiation in the Cucurbita pepo complex: C. pepo var. medullosa vs. C. texana. Econ. Bot. 39:289-299.
- OECKER, O.S. 1985. Numerical analysis of allozyme variation in Cucurbita pepo. Econ. Bot. 39:300-309.
- OECKER, O.S. and WILSON, H.O. 1986. Numerical analysis of seed morphology in Cucurbita pepo. Syst. Bot. 11:595-607.
- OECKER, O.S. and WILSON, H.D. 1986. Allozyme variation in the Cucurbita pepo complex: C. pepo var. ovifera vs. C. texana. Syst. Bot. 12:263-273.

14.074 CRISO132023 BIOCHEMICAL GENETICS OF HOST-PATHOGEN INTERACTIONS

MAGILL C; Plant Pathology & Microbiology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX06890 Project Type: HATCH Agency IO: CSRS Period: 19 JUN 87 to 31 MAY 92

Objectives: THE LONG TERM GOAL OF THIS PROJECT IS TO UNDERSTAND THE GENETIC BASIS FOR MOLECULAR EVENTS THAT OCCUR IN THE INTERACTION BETWEEN PLANTS AND POTENTIAL PATHOGENS THAT LEAO EITHER TO OISEASE OR RESISTANCE. ULTIMATELY THE ABILITY TO IDENTIFY AND CLONE GENES INVOLVEO IN HOST RESISTANCE OR TOLERANCE WILL PERMIT THE USE OF GENETIC ENGINEERING TECHNOLOGY TO PRODUCE IMPROVED VARIETIES. SPECIFIC OBJECTIVES ARE: 1) TO DEMONSTRATE GENE ACTIVATION IN CORN AND RICE EXPOSED TO FUNGAL PATHOGENS, 2) TO CLONE GENES WHICH ARE INVOLVED IN HOST OFFENSE, 3) TO OFTERMINE IF PHENYLPROPANDIO SYNTHESIS PATHWAYS PLAY A ROLE IN RESISTANCE OR TOLERANCE IN CEREALS, 4) TO OETERMINE IF OEFENSE RESPONSE GENES ACCOUNT FOR SINGLE GENE RESISTANCE, MULTIGENIC TOLERANCE, OR HYPERSENSITIVE RESPONSE.

Approach: THE TIMING AND LEVELS OF NEW PROTEINS SYNTHESIZED FOLLOWING INOCULATION OF CORN WITH RUST AND RICE WITH BLAST WILL BE ESTABLISHED ON 2 O GELS. MESSENGER RNA ISOLATED FROM COMPATIBLE AND INCOMPATIBLE INTERACTIONS WILL BE USED TO MAKE CONA LIBRARIES. OIFFERENTIAL HYBRIDIZATION WILL BE USED TO SCREEN EACH LIBRARY FOR UNIQUE MRNAS; THUS IDENTIFYING GENES THAT MAY FUNCTION IN VIRULENCE,

RESISTANCE, OR TOLERANCE, ENZYME ACTIVITY AND NORTHERN BLOTS WILL BE USED TO IDENTIFY AND QUANTITATE EXPRESSION OF PHENYLPROPANOID PATHWAY GENES, AND SOUTHERN HYBRIDIZATION WILL BE USED TO DETERMINE THE HOST OR PATHOGEN ORIGIN OF SPECIFIC CDNAS.

Progress: 88/01 to 88/12. Time-course studies of the interaction between maize and Puccinia polysora over the first 36 hours of infection do not reveal any differences at the microscopic level between B37R, a resistant line, and the near-isogenic susceptible host, B37. Though new proteins are produced in both lines within 14 hours of inoculation, we have not been able to identify the proteins or enzymes involved. Of several PAL pathway enzymes examined for total activity changes or isozymes, only peroxidase shows differences in the appearance of isozyme bands, but not until later than 36 hours. Auxotrophic mutants have been isolated in three strains of Magnaporthe grisea (Pyricularia oryzae), the causal organism of rice blast. Two of the lines are sister strains with allelic differences for at least four linked loci as identified by host-differential tests. In addition, one is stable and the other is not. The third strain is an isolate from Guyana which is heterokaryon-compatible with the others, and was chosen to provide a potential source of variation at the DNA level. Several probes from other organisms have been tested for use as homologous probes, and the tryp-1 probe from Neurospora has revealed a restriction fragment polymorphism. The six chromosomes of P. oryzae have been separated by transverse alternating field electrophoresis, and the tryp-1 chromosome identified.

Publications: 88/01 to 88/12

LIVORE, A.B., GRUBB, P., MAGILL, C.W. and MAGILL, J.M. 1988. Sephadex column procedure for DNA isolation is also useful for detecting dsRNA. Nucl. Acids Res. 16:776.

MAGILL, J.M. and MAGILL, C.W. 1988. DNA Methylation in Fungi. Developmental Genetics. (In press.)

LIVORE, A.B. 1988. Characterization of chloroplast DNA in androgenic albino rice plants. Ph. D. Dissertation. TAMU, College Station. 77p.

14.075 CRISO142057 GENOTYPIC CHARACTERIZATION OF HETEROTIC CORN POPULATION USING RFLP'S

STUBER C W: HELENTJARIS T; Native Plant Institute, Salt Lake City, UTAH 84112. Proj. No.: 0101-21020-009-14S

Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: O1 APR 87 to 30 SEP 89

Objectives: Characterize genotypically lines from the heterotic corn hybrid, B73 X Mo17, and lines from a contributor hybrid, Oh43 X Tx303, for RFLP's.

Approach: About 320 F(3) lines from the corn hybrid, B73 X Mo17, and about 230 F(3) lines from the hybrid, $Oh43 \times T \times 303$ will be

characterized for their RFLP genotypes by using markers distributed appropriately throughout the genome. Results from these characterizations will be combined with isozyme characterizations and quantitative trait data from field studies to identify and locate genetic factors that contribute to the heterotic response in the B73 X Mo17 hybrid. Funded from Competitive Grant 0101-21020-008-84R.

29

Progress: 88/01 to 88/12. RFLP genotyping of 216 F3 lines from the Oh43 X Tx303 cross was completed. Field and genetic marker information from these lines, from F3 lines developed from the B73 X Mo17 hybrid, and from respective backcrosses and testcrosses was used to identify genetic factors (or chromosomal segments) from Oh43 and Tx303 that might be transferred to Mo17 and B73, respectively, to enhance the heterotic response of the B73 X Mo17 hybrid. Marker-facilitated backcrossing is now being used to transfer the appropriate factors. 1200 backcross families (600 to each of the lines, B73 and Mo17) were genotyped with RFLP and isozyme markers. Selected families were used to generate the third cycle of backcrossing.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

14.076 CRISO099212 MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS

OSBORN T C; Agronomy; University of Wisconsin, Madison, WISCONSIN 53706. Project Type: HATCH Proj. Nc.: WISO3068 Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 90

Objectives: The overall objective is to obtain molecular evidence for the generation of new genetic variation in agronomic crops. The specific objectives are to 1) characterize changes in the copy number of repeated DNA sequences in maize populations under selection and in alfalfa lines derived from tissue culture, 2) determine if changes in repeated DNA are associate with phenotypic variation and 3) study the inheritance of variation in repeated DNA copy number.

Approach: Genomic DNA libraries of a selected maize population and of an alfalfa line used for tissue culture regeneration will be screened by differential hybridization using the DNAs isolated for cloning and DNAs from a divergently selected population (maize) or tissue culture derived variants (alfalfa). Differentially hybridizing DNA clones will be used as probes on slot blots and Southern blots containing DNAs of the starting inbred lines and the intermediate and advance selections (maize) or the parent and tissue culture derived lines (alfalfa). Changes in the copy number of repeated DNA sequences that are correlated with phenotypic changes will be analyzed in segregating progenies of maize and alfalfa. Thus the inheritance of repeated DNA copy number and its relationship to new phenotypic variation will be determined.

Progress: 88/01 to 88/12. This is a summary of progress in the molecular characterization of genetic variation in three different plant systems. The first system deals with new genetic variation that arises in alfalfa tissue culture. We have identified several repeated DNA sequences that have changed copy number in somaclonal variants. These changes occur readily in culture and they maybe associated with phenotypic variation arising in the culture process. These sequences have been cloned and the clones provide tools for further investigations on the molecular origins of somaclonal variation. The second system is the organization and expression of genes encoding arcelin seed protein in common bean. We have backcrossed arcelin genes from wild into cultivated beans and expression of arcelin in these lines confers high levels of bruchid beetle resistance. We have partially characterized alleltic variants of arcelin to determine their relationship to each other and to the related seed protein, phytohemagglutinin (PHA). Arcelin genes appear to have arisen by duplication of PHA genes to create a complex gene family. The third system involves genome characterization in Brassica species. We have used molecular markers (RFLPs) to study evolutionary relationships and to construct genetic linkage maps. These markers also are being used to identify and characterize genes controlling morphological variation.

Publications: 88/01 to 88/12

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- OSBORN, T.C., ALEXANDER, O.C., SUN, S.S.M., CAROONA, C. and BLISS, F.A. (1988)
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- OSBORN, T.C., BUROW, M. and BLISS, F.A. (1988) Purification and characterization of arcelin seed protein from common bean. Plant Physiol. 86:399-405.
- SLONG, K.M., OSBORN, T.C. and WILLIAMS, P.H. (1988) Brassica taxonomy based on nuclear restrication fragment length polymorphisms (RFLPs) 1. Genome evolution of diploid and amphidiploid species. Theor. Appl. Genet. 75:784-794.
- SONG, K.M., OSBORN, T.C. and WILLIAMS, P.H. (1988) Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs) 2. Preliminary analysis of subspecies within B. rapa (syn. Campestris) and B. oleracea. Theor. Appl.

14.077 CRISO027450 ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE

KERMICLE J L; Genetics; University of Wisconsin, Madison, WISCONSIN 53706.

Proj. No.: WISO1489 Project Type: STATE Agency IO: SAES Period: 01 OCT 70 to 30 JUN 91

Objectives: The R chromosome region in maize is under study with a view to determining the number, kind, and arrangement of the genic and structural components involved inthe control of anthocyanin pigmentation.

Approach: Organization of the region is investigated by analysis of the variation found in cultivated races or that arising in experimental cultures. Variation among races appears to reflect local chromosome structural differentiation, such as small duplications or inversions, as well as genic divergence. Changes arise in culture spontaneously by mutation or intralocus recombination, and are induced in appropriate heterozygotes by paramutation. The classification in intralocus recombinants for outside markers permits recognition and serial ordering of the various R components. Characterization for simulateous changes in less conventional properties, such as paramutation, permits the corresponding determiners to be spatially ordered relative to the pigmenting elements. Particular attention also is to be given to the following phenomena: Interaction between determinants based on local position; suppression by particular R alleles of chlorophyll striping; a possible effect of paramutation on frequency of recombination in the R region; and the analysis of R mutations not associated with exchange of linked markers.

Progress: 88/01 to 88/12. The R gene regulates the distribution and timing of anthocyanin pigmentation, plant part by plant part. A collection of 40 mutations induced by insertion of the transposable element Oissociation in an R-allele that confers strong seed color (Sc) is being used to focus on the difference between (Sc) and an allele that confers pigmentation only on vegetative tissues. (P). Recombinational analysis of the (Sc) mutants with (P) identify two classes. The 24 mutants mapping to the telomere side of the gene recombine with (P) to restore (Sc) function, indicating that a segment of (P) can substitute for (Sc) at the site of insertion and distally within the gene. Os was transferred from (Sc) to (P) in the reciprocally marked recombinant class, inciting plant color mosaicism in the three cases tested. Function of (Sc) involving the 16 mutants mapping to the centromere side of the gene is not restored through crossing over with (P). The components responsible for tissue-specific difference in (Sc) and (P) action reside in this region. The corresponding segments have been cloned molecularly and the nucleotide sequence is being determined in order to pinpoint the critical differences between (Sc) and (P). Supported by OOE Grant OE-FG02-86ER13539 and NSF Grant OMB-8719615.

Publications: 88/01 to 88/12

DELLAPORTA, S.L., GREENBLATT, I.M., KERMICLE, J., HICKS, J.B. and WESSLER, S.

1987. Molecular cloning of the maize R-nj allele by transposon tagging with Ac, Stadler Symposium 18:263-282.

KERMICLE, J.L. 1988. Recombinant mutable alleles of the maize R gene. In: Plant Transposable Elements, pp. 81-89, O.E.

Nelson and E.T. Bingham, eds. KERMICLE, J.L., ALLEMAN, M. and DELLAPORTA, S.L. 1989. Sequential mutagenesis of a maize gene using the transposable element Dissociation. Genome, in press.

14.078 CRIS0032340 MUTANT GENES THAT AFFECT ENDOSPERM DEVELOPMENT IN MAIZE

NELSON O E; Genetics; University of Wisconsin, Madison, WISCONSIN 53706.

Proj. No.: WISO1627 Project Type: HATCH

Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 88

Objectives: To attempt to further our understanding of endosperm development in maize with a continuing investigation of the mutant genes affecting starch and protein biosynthesis.

Approach: The enzymic deficiencies in the numerous mutants that affect starch synthesis in maize have been elucidated in only a limited number of mutants. Efforts in this area will contiue with particular emphasis on the initiation of starch synthesis. Some mutants that affect the synthesis of storage proteins also apparently affect nitrogen assimilation in the sporophyte and these will be investigated.

Progress: 84/01 to 88/12. The transposable element research concentrated on bz-ml3 and the changes derived therefrom. The 1986 report detailed the nature of the unusual processing event that enable the high level expression of a Bz allele, bz-ml3 (CS9), with a dSpm insertion in an exon of the gene. Since CS9 resulted from an internal deletion in the dSpm present in bz-ml3 but has ten-fold higher amounts of the gene product, we have tried to ascertain the reason. it is that the deletion in the bz-ml 3 dSpm that created CS9 removed an internal splice acceptor that is used by the terminal acceptor site for the remainder. The use of this internal site leads to a message with a translation stop codon in the portion of the dSpm that remains in the message while the use of the terminal acceptor yields an in-frame message that is translated to give a functional enzyme. Examination of the starch-synthesizing maize mutant, dull (du) makes it likely that this mutant has very low levels of an enzyme that carries out the following reaction, Glucose-1-P + ATP = Glucose-1, 6-bisP + ADP. The mutant effect, which is a reduction in the amount of amylopectin synthesized, which leads to an increase in the proportion of the starch that is amylose, may be a result of a shortage of Glc-1,6-bisP. In addition to being a cofactor for phosphoglucomutase, we believe that Glc-1,6-bisP is incorporated into the growing starch chain where it forms a branch point.

Publications: 84/01 to 88/12

SCHIEFELBEIN, J.W., RABOY, V., KIM, H.Y. and NELSON, O.E., JR. 1988. Molecular characterization of Supressor-mutator (Spm)-induced mutations at the bronze-1 locus im maize: the bz-ml3 alleles. In "Plant Transposable Elements." SCHIEFELBEIN, J.W., FURTEK, D.B., DOONER, H.K. and NELSON, E.E. 1988. Two mutations in a maize bronze-1 allele caused by transposable elements of the Ac-Ds family alter the quantity and quality of the gene product. Genetics 120:767-777.

KLEIN, A.S., CLANCY, M., PAJE MANALO, L., FURTEK, D.B., HANNAH, L.C. and NELSON, D.E. 1988. The mutation bronze-mutable 4 Derivative 6856 in maize is caused by the insertion of a novel 6.7 kilobase pair transposon.

FURTEK, D.B., SCHIEFELBEIN, J.W., JOHNSTON, F. and NELSON, O.E. 1988. Sequence comparisons of three wild-type Bronze-1 alleles from Zea mays. Plant Mol. Biol. 11:473-481.

14.079* CRISO097400 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY

JOHNSON J; ROTH D; BULLA L; Molecular Biology; University of Wyoming, Laramie, WYOMING 82070. Proj. No.: WYO-224-86 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To isolate and to characterize genes in beneficial and pathogenic microbes controlling nitrogen fixation, biological control of pathogens and weeds, host-parasite interactions, host specificity and hypovirulence.

Approach: Although research groups participating in this project are working on different problems and with different organisms, they are all using the same tools of modern molecular genetics and recombinant DNA technology. Before discussing specific procedures which will be used to clone and to characterize genes in plants and associated microbes, it would be useful to briefly discuss the potential and limitations of some of these procedures and techniques.

Progress: 88/01 to 88/12. All projects being carried out have shown substantial progress. Johnson; Frankia N(subscript 2) Fixation-Fragments of Frankia DNA which activate transcription of a LUX cassete have been identified and DNA sequence analyses being done to identify promoter elements. RothJohnson; antisense RNA inhibition of plant virus. Sequences complementary to the 5' region of TMV have been tested in vitro and in vivo for the ability to interfere with TMV gene expression. Roth; TMV replicase activity/protein kinase activities. A unique protein kinase induced by plant viroids has been identified. Purification and characterization of TMV induced replicase is in progress. Bulla-characterization of B. thuringiensis insecticidal proteins. The genes encoding the insecticidal activity are being

cloned into the blue-green algae Anacystis nidulans to develop a self-perpetuating biological insecticide effective against insect vectors of disease.

Publications: 88/01 to 88/12
 CRUM, C.J., JOHNSON, J., NELSON, A. and ROTH,
 D. (1988). Nucl. Acids Res. 16, 4569-4581.
 HIDDINGA, H.J., CRUM, C.J., HU, J., and ROTH,
 D. (1988). Science 241, 451-453.

14.080 CRISO140327
ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM
YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES

STUBER C W; GERIC I; Faculty of Agriculture; Inst of Field & Veg Crops, Novi Sad, YUGOSLAVIA Proj. No.: 8004-21220-127-00P

Project Type: GRANT

Agency ID: OICD Period: 01 SEP 85 to 31 AUG 90

Objectives: 1) To conduct a survey of isozyme variation among maize populations from Yugoslavia and other European countries; B) using isozyme data, to group these populations on the basis of genetic similarities using distance and cluster analyses; C) To relate these data to data from similar studies of maize from North and South America.

Approach: Isozyme (allozyme) genotypes will be scored on a representative sample of plants from maize populations from Yugoslavia and other European countries. With the use of appropriate numerical taxonomic techniques, including genetic distance measures and cluster analyses, evolutionary history, migration patterns, and degrees of diversity will be investigated for these European populations. In addition, these data will be combined with data acquired at Raleigh, NC on North American and Latin American maize populations to provide a comprehensive evaluation of genetic diversity and relationships of maize germplasm throughout much of the world.

Progress: 88/01 to 88/12. Sixty populations of maize representing different regions in Italy were assayed electrophoretically for frequencies of isozyme alleles for 20 isozymic loci. Detected alleles found were similar to those found in Yugoslavian populations in earlier assays, with only two new alleles found in Italy. Frequencies of the alleles in Italian populations differ from the Yugoslavian populations, however, which indicates that the Italian populations were genetically different from those assayed from Yugoslavia. Studies to be made in the next research period will be conducted on maize populations, largely flints, representing different regions of France.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

CM 15 GRAIN SORGHUM

15.001* CRIS0047923 GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM

CHOUREY P S: NIBLETT C L; Agricultural Research Service; University of Florida, Gainesville, FLORIDA 32611. Proj. No.: 6615-22000-001-01S

Project Type: COOPERATIVE AGREE.

Period: 01 OCT 82 to 30 SEP 87 Agency ID: ARS

Objectives: To elucidate gene regulation & expression & to map molecular distributions at the cellular and organismal levels in maize and sorghum.

Approach: Specific parts of the genomes of maize & sorghum will be cloned through re-combinant DNA techniques. These will then be used as probes in DNA: DNA hy- bridization experiments to enable identification of functional roles of specific DNA's.

Progress: 87/01 to 87/09. A region of the maize T cytoplasm mitochondrial DNA (mtDNA) associated with male sterility and disease susceptibility was identified and mapped. The gene atp-6 and co-transcribed genes urf-13-T and ORF25 share promoters by virtue of a 5 Kb repeated region 5' to the genes. Urf13-T was deleted or truncated in all tissue culture-derived mutants to male fertility and disease resistance. The genes atp 6 and ORF 25 were unaffected in the mutants. Antibody to a synthetic peptide, derived from the DNA sequence of urf13-T, immunoprecipitated a 13 kD polypeptide, identifying it as the gene product. The 13 kD polypeptide was absent in all mutants.

Publications: 87/01 to 87/09

TABEIZADEH, Z., PRING, D.R. and VASIL, I.K. 1987. Analysis of mitochondrial DNA from somatic hybrids of Saccarum officinarum (sugarcane) and Pennisetum americanum (pearl millet). Plant Molec. Biol. 8:509-513.

OZIAS-AKINS, P., PRING, D.R. and VASIL, I.K. 1987. Rearrangement in the mitochondrial genome of somatic hybrid cells of Pennisetum americanum (L.) K. Schum. Panicum maximum Jacq. Theor. Appl. Genet. 74:15-20.

SMITH, R.L., CHOWDHURY, M.K.U. and PRING, D.R. 1987. Mitochondrial DNA rearrangements in Pennisetum assoc. with reversion from cytoplasmic male sterility to fertility. Plant Molec. Biol. 9:277-286.

CRISO097156 COSMID MAPPING OF MITOCHONDRIAL DNA OF MALE FERTILE AND MALE STERILE SORGHUM

PRING D R; Plant Pathology; University of Florida, Gainesville, FLORIDA 32611. Proj. No.: FLA-PLP-02525 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 85 to 31 AUG 88

Objectives: Proj 8500310. Study the genomic organization and structure of mitochondrial DNA from male-fertile and male-sterile sorghum and locate coding sequences of selected genes.

Assemble maps of gene regions.

Approach: Construct cosmid clone libraries of kafin and IS1112C mitochondrial DNA. Using colony hybridization and Southern blots. hybridize selected coding sequences, isolate and map each gene on the two libraries. Extend maps of each coding sequence by cosmid mapping of overlapping cosmids. Assemble, cosmids into maps, and compare male-fertile and male-sterile mitochondrial DNA.

Progress: 88/01 to 88/12. The organization and relationships among sorghum mitochondrial genomes were elucidated through cosmid mapping of a male fertile kafir cytoplasm (Btx393) and a group A3 cytoplasm (IS1112C), which is male-sterile, ca. 300 kb of IS1112C has been mapped with five enzymes using probes for cox1, II, and III, atpA, 6, and 9, cob, 18S and 26S rDNA, and rbcl. Maps of Kafir mtDNA for five of these genes are co-linear, with variation in recombinationally active repeats observed between the two cytoplasms. The genome carries numerous sequences with participate in recombination. Among five repeats in IS1112C identified are regions adjoining 18\$ rDNA, 26\$ rDNA, atp6, and cox1. One configuration of the master chromosome positions cox11, atp9, atp6, and cox1. One configuration of the master chromosome positions cox11, atp9, atp6, and coxl within a 55 kb region. The placements of cox11, apt9, and atp6 are unambigous, but two points of recombination are found between atp6 and cox1. Tentative distances are 9 kb from cox11 to atp9, 11 kb from atp9 to atp6, and 30 kb from atp6 to cox1. Sorthum cyroplasms, male fertile/male sterile, differ in occurrence of repeated sequences, as identified by genomic probing with selected fragments. For example a repeat adjacent to atp6 in IS1112C is apparently single copy in certain other cytoplasms, while other repeats occur as two copies in all cytoplasms examined. Other probes from repeated regions reveal a segregation of genomic configurations into groups.

4

Publications: 88/01 to 88/12 PRING. D.R., GENGENBACH, B.G. and WISE, R.P.

1988. Recombination is associated with polymorphism of the mitochondrial genomes of maize and sorghum. Phil. Trans. R. Soc. Lond. B 319:187-198.

CRIS0089829 15.003* CELLULAR AND MOLECULAR GENETICS FOR CROP **IMPROVEMENT**

PRING D R; CHOUREY P S; HIEBERT E; Plant Pathology; University of Florida, Gainesville, FLORIDA 32611.

Proj. No.: FLA-PLP-02317 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 82 to 30 SEP 88

Objectives: Identification and Characterization of Agriculturally Important Genetic Systems. Regulation of Gene Expression and The Delivery of Genetic Material to Higher Plants and Associative Microorganisms. Somatic Cell Genetics and Plant Development: The Modification, Selection Regulation, and Propagation of Plants through Cell and Tissue

Culture.

Approach: Plant viral genomes will be mapped and their products characterized. Recognition factors, both host and bacterial, will be characterized. Transposable elements of maize will be used as specific mutagens to identify rate limiting steps in starch biosynthesis. Plasmid-like DNAs in maize and sorghum will be tested as genetic vectors. Plant regeneration from protoplasts and callus will be attempted via organogenesis and somatic embryogenesis.

Progress: 83/10 to 88/09. Promoters and processing sites of maize mitochondrial atp6 are positioned 5' to Turf-13 and ORF221 in T cytoplasm maize, providing regulatory sequences associated with the 13 kD gene product of Turf-13. Maize nuclear backgrounds influence abundance of at least five transcripts associated with the gene. Abundance of the 13 kD gene product is reduced dramatically, while the Rf1 restorer only slightly reduces abundance of major transcripts, suggesting a role of the gene in translation. A maize cell suspension culture was used to study the biology and replication of mitochondrial DNA (mt DNA) and the two minicircular DNAs. All mt DNAs were synthesized rapidly during logarithmic growth phase, whereas no synthesis could be detected in stationary phase. The minicircular DNAs replicated earlier than the principal mt DNA. These data indicate that components of mitochondrial genome exhibit differential replication. Restriction digestion and Southern blot analyses of the bean golden mosaic virus (BGMV) isolated in Florida in comparisons with the Puerto Rican BGMV isolate revealed a high degree of sequence homology between the two isolates. However, distinct restriction patterns with four different endonucleases indicate that the isolates are not identical at the genomic level. A monoclonal antibody prepared to the Florida BGMV was useful in distinguishing some biological variants of BGMV.

Publications: 83/10 to 88/09

- KENNELL, J.C., WISE, R.P. and PRING, D.R. Influence of nuclear background on transcription of a maize mitochondrial region associated with Texas male sterile cytoplasm. Mol. Gen. Genet. 210:399-406. 1987.
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- GILBERTSON, R.L., FARIA, J.C., HIEBERT, E. and MAXWELL, D.P. 1988. Properties and cytology of bean golden mosaic in Brazil. Phytopathology, Abst. 441 submitted for the Annual Meetings of APS, San Diego, CA, Nov. 13-17, 1988.
- CHANG, C.A., HIEBERT, E. and PURCIFULL, D.E. 1988. Characterization and immunological analysis of nuclear inclusions induced by bean yellow mosaic and clover yellow vein

potyvirusus. Phytopathology 78, in press. CHANG, C.A., HIEBERT, E. and PURCIFULL, D.E. 1988. Analysis of in vitro translation of the bean yellow mosaic virus RNA and the inhibition of proteolytic processing by antiserum to the 49K nuclear inclusion protein. J. gen. Virol.

HIEBERT, E. and DOUGHERTY, W.G. 1988.
Organization and expression of the vira.

15.004* GENETICS AND PHYSIOLOGY OF FUSARIA

CRIS0095292

LESLIE J F; Plant Pathology; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KANO0547 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: Characterize natural populations of Fusarium moniliforme with respect to mating groups, mating types, vegetative incompatibility groups, meiotic mutants and DNA restriction fragment length polymorphisms, and measure correlations of these traits with pathogenicity. Define the overall regulation of nitrogen catabolism in Fusarium roseum 'Graminearum' and test both naturally occurring variants and mutants induced in the laboratory for nitrogen catabolic activity, pathogenicity and the production of mycotoxins.

Approach: F. moniliforme isolates from corn and sorghum will be subdivided on the basis of their race, mating-type and vegetative incompatibility group. Representatives of each class will be tested for pathogenicity in a corn seedling system, and for the presence of meiotic mutants and DNA restriction fragment length polymorphisms. Nitrogen catabolic activity in mutant and wild-type strains of F. roseum 'Graminearum' will be measured by dry weight of cultures grown in shake flasks, linear growth rates in race tubes, resistance to common nitrogen analogs and defects in enzymes such as nitrate reductase, glutamate dehydrogenase and glutamine synthetase. Mutants defective in nitrogen catabolism will be tested for pathogenicity in a corn seedling system.

Progress: 88/01 to 88/12. We have completed classical genetic studies of nitrate metabolism in F. moniliforme. We mapped all seven nit mutants with respect to each other and tested nitrate reductase levels of these mutants (no activity was detected). nit1 and nit3 mutants were recovered most frequently, but the relative frequencies with which the mutants occurred could be altered by changing the nitrogen source in the minimal chlorate medium. Different strains throw chlorate sectors at different frequencies. These frequency differences are heritable as a quantitative trait and are consistent with a transposable element as the cause for the sectoring phenomenon. A series of five crn mutants (chlorate-resistant, utilize nitrate) were also characterized. All of these mutants had detectable nitrate reductase activity, and two of them appear to be allelic with different nit mutants. The other three loci may be involved in regulation or in nitrate uptake. We have continued to characterize populations of F.

moniliforme using vegetative compatibility as our measure of variability. At least 40 different vegetative compatibility groups (VCGs) have now been identified. In general F. moniliforme populations are highly variable with respect to this trait. We have also described a novel phenomenon - termed "heterokaryon self-incompatibility" - which can give false negatives in complementation tests; in our studies this trait is under the control of a single nuclear gene.

Publications: 88/01 to 88/12

KLITTICH, C.J.R. and LESLIE, J.F. 1988. Nitrate reduction mutants of Fusarium moniliforme (Gibberella fujikuroi). Genetics 118:417-423.

KLITTICH, C.J.R., CORRELL, J.C. and LESLIE, J.F. 1988. Inheritance of sectoring frequency in Fusarium moniliforme (Gibberella fujikuroi). Experimental Mycology 12:289-294.

KLITTICH, C.J.R. and LESLIE, J.F. 1988.

Multi-well plates for complementation tests of Fusarium. Fungal Genetics Newsletter 34:21-22.

KLITTICH, C.J.R. and LESLIE, J.F. 1988. Chlorate-resistant, nitrate-utilizing mutants of Fusarium moniliforme (Gibberella fujikuroi). Journal of General Microbiology (in press).

15.005* CRISO076599
BIOLOGY, EPIDEMIOLOGY & CONTROL OF VIRUSES &
MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF
CORN

LOMMEL S; Plant Pathology; Kansas State University, Manhattan, **KANSAS** 66506.

Proj. No.: KANOOO9 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 82 to 30 SEP 88

Objectives: Identify and characterize viruses, spiroplasmas and MLO's occurring in corn and sorghum in the United States and to develop methodology suitable for virus detection in epidemiological studies.

Approach: conduct statewide surveys; collect tissue samples for analyses. Produce viral antisera and develop serological test procedures. Establish host range differential for virus identification. Characterize biological and serological properties of viral agents. Establish field plots and inoculate at different plant growth stages and evaluate for symptomatology and yield. Screen crop genotypes for sources of resistance to viral agents; study effect of cultural practices on disease epidemiology.

Progress: 78/07 to 88/09. The complete genome of maize chlorotic mottle virus (MCMV) has been cloned and sequenced. The genome organization and replicative strategy of the virus has been determined. In collaboration with Dr. R.C. Nutter at Oklahoma State University, the in vitro synthesis of various gene products is being determined. An infectious transcription system for MCMV is currently being developed to perform precise structural and functional mutations.

Publications: 78/07 to 88/09

LOMMEL, S.A., KENDALL, T.L., SIU, N.F. and NUTTER, R.C. 1988. cDNA cloning and in vitro translation of the maize chlorotic mottle virus genome. J. Gen. Virol. (Submitted).

NUTTER, R.C., SHEETS, K., PANGANIBAN, L.C. and LOMMEL, S.A. 1989. The complete nucleotide sequence of the maize chlorotic mottle virus genome. Virology (In preparation).

15.006 CRISO133186 ISOZYME GENETICS OF SORGHUM BICOLOR AND RELATED SPECIES

DOEBLEY J F; Botany; University of Minnesota, St Paul, **MINNESOTA** 55108.

Proj. No.: MINR-8702803 Project Type: CRGO Agency ID: CRGO Period: 15 SEP 87 to 30 SEP 89

Objectives: PROJ. 8702803. To determine genetic control and subcellular localization of sorghum isozymes; to determine linkage relationships of the isozyme genes; to survey genetic variation among a broad array of cultivated races of sorghum and their wild relatives.

Approach: These objectives will be accomplished through the use of starch gel electrophoresis and histochemical staining techniques.

Progress: 88/01 to 88/12. During the past year, we have analyzed 30 additional F(2) families for segregating isozymes. Altogether now we have segregation data for 24 of the 32 loci understudy. Two additional loci will be analyzed this coming year. Thus far we have found 5 linkage groups involving 12 loci. Two of these linkage groups are the same as in maize, suggesting that the chromosomal segments on which these genes are borne have been conserved since the divergence of maize and sorghum. During the coming year we will complete our genetic and linkage analyses. This year we also analyzed 50 additional U.S. breeding lines and 50 accessions from the world collection as part of our effort to determine the amount and distribution of genetic variation in sorghum. These data show a decrease in variation as one goes from wild species to landraces to U.S. breeding lines. We have also analyzed several species of wild sorghum with chromosome numbers of n=5, 10 and 20. These analyses showed that the n=5 sorghums have approximately the same number of isozyme genes as the n=10 sorghums. This suggest that many of the duplicate isozyme loci in the polyploid (n=10) sorghums have been silenced since their derivation from the n=5 diploids.

Publications: 88/01 to 88/12

MORDEN, C.W., DOEBLEY, J.F. and SCHERTZ, K.F. Allozyme variation in the Old World races fo Sorghum bicolor (Poaceae). Amer. J. Bot. (in press).

DOEBLEY, J., WENDEL, J., SMITH, J., STUBER, C. and GOODMAN, M. 1988. The origin of Cornbelt maize: the isozyme evidence. Econ. Bot. 42:120-131.

MORDEN, C., DOEBLEY, J. and SCHERTZ, K. 1988. Genetic control and subcellular localization of aconitase isozymes in Sorghum. Journal of Heredity 79:294-299. DOEBLEY, J. Isozymic evidence and the evolution of crop plants. 1988. In D. Soltis and P. Soltis (eds.) Isozymes in Plant Biology (in press).

15.007 CRISO132699 STRUCTURAL ANALYSIS AND TRANSCRIPTION OF TRNA GENES FROM HIGHER PLANTS

MA D P; Biochemistry; Mississippi State University, Mississippi State, **MISSISSIPPI** 39762.

Proj. No.: MIS-1507 Project Type: HATCH Agency ID: CSRS Period: 01 SEP 87 to 30 JUN 92

Objectives: To isolate and characterize cytoplasmic tRNAs and their genes from Sorghum bicolor. To dissect the DNA control elements for plant tRNA gene transcription.

Approach: Isolate and purify cytoplasmic tRNAs and use them as probes to screen a sorghum genomic library constructed in a lambda Charon 35 vector. Characterize and sequence the inserts of lambda recombinant clones containing tRNA genes. Use the isolated and modified tRNA genes for in vitro transcription experiment.

Progress: 88/01 to 88/12. Chlamydomonas reinhardtii mitochondrial DNA was isolated. digested with the HindIII restriction enzyme, and shotgun cloned into the HindIII site of M13mp18 vector. One of the recombinant M13 clones with 1.65kb DNA insert which contains part of the ND2 gene was further characterized and sequenced using the dideoxy chain termination method. Besides encoding the 3' end of the ND2, this HindIII fragment contains a ND6 gene that encodes the subunit 6 of NADH dehydrogenase. This polypeptide of 162 amino acids has a strong preference for using some genetic condons which are also consistently found in the Chlamydomonas mitochondrial proteins. Nineteen base pair downstream from the 3' end of ND6 sequence is a gene coding for tRNATrp based on its anticodon sequence. The tRNATrp gene represents the first tRNA sequence found in the Chlamydomonas mitochondrial DNA. Like most other eukaryotic tRNA genes, the 3 terminal CCA sequence of the mature tRNA transcript is not encoded by the tRNATrp gene. The rT(U)-U-C-Pur-A in loop IV which occurs in other non-organelle elongator tRNAs is also conserved. Both the genes for ND6 and tRNATrp are transcribed in the same direction. The close-linked protein and tRNA genes strongly suggest that the mitochondrial RNA processing model is present in the Chlamydomonas mitochondria as well.

Publications: 88/01 to 88/12
PETTIGREW, D.W., MA, D.P., CONRAD, C.A. and
JOHNSON, J.R. 1988. E. coli glycerol
kinase:cloning and sequencing of the glpk
gene and the primary structure of the
enzyme. J. Biol. Chem. 263:135-139.

MA, D.P. and YANG, Y.W. 1988. Nucleotide sequence of a tRNA_G_l_y gene for Sorghum bicolor. Nucl. Acids Res. 16:3588-3588. YAMAMOTO, T., WATKINSON, I.A., KIM, L., SAGE, M.V., STRATTON, R., AKANDE, N., LI, Y., MA, D.P. and ROE, B.A. 1988. Nucleotide sequence of the gene codeing for a 130-kDa mosquitocidal protein of Bacillus thuringiensis israelensis. SALIN, M.L., DUKE, M.V., OESTERHELT, D. and MA, D.P. 1988. Cloning and determination of the nucleotide sequence of Mn-containing superoxide dismutase gene from Halobacterium halobium. Gene 70:153-159. MA, D.P., YANG, Y.W. and HASNAIN, S.E. 1988. Nucleotide sequence of Chlamydomonas reinhardtii mitochondrial genes coding for subunit 6 of NADH dehydrogenase and tRNA_T_r_p. Nucl. Acids Res. 16:11373-11373. MA, D.P., YANG, Y.W. and HASNAIN, S.E. 1989. Nucleotide sequence of Chlamydomonas reinhrdtii mitochondrial genes coding for

15.008 CRISO133059 FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR IDENTIFICATION AND CONTROL OF INSECTS

tRNA_G_1_n (UUG) and tRNA_M_e_t (CAU).

Nucl. Acids Res. "In press".

JOHNSON J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6916 Project Type: HATCH Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

Objectives: 1) Develop flow cytometry and in particular total nuclear DNA content determination as a cytotaxonomic tool, for the recognition of host races, biotypes, and crytic species of insects. 2) Catalogue the nuclear DNA content of insects of economic interest. 3) Develop multiparameter DNA flow cytometry using dyes which are specific for total DNA, adenine and thymine rich repetitive DNA, and guanine and cytosine rich repetitive DNA. 4) Demonstrate the developmental pattern and mode of inheritance of DNA content variation for both euchromatic (unique) and heterochromatic (repetitive) insect DNA sequences. 5) Develop methodology to sort insect nuclei and chromosomes as an aid to gene cloning in insects.

Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

Progress: 88/01 to 88/12. We have now had one year of work with this unique new methodology for the study of heritable variation in insects. We have measured the DNA content of over 1500 individual fire ants including 3 native and two imported species. In addition, we have measured the DNA content of over 200 honey bees, nearly 300 boll weevils, plus smaller number of individuals from 10 other species. The results have been unexpected and exciting. In particular we find: DNA content is a diagnostic tool that aids in

identification of species, populations and types. To date, very significant and predictable ONA content differences have been found between 5 fire ants species, between 4 species of boll weevil, and between the Africanized form of the honey bee, the European honey bee and the F-1 hybrid between the two forms. DNA content variation exists within and among some populations. The most extreme variation we have observed occurs in a fire ant population in Walton Co., Georgia, were 129 of 515 males, females, and workers show 3N or triploid DNA amounts. The remainder are diploid or haploid as expected. The smallest significant variation occurs between populations of the boll weevil Anthonomus grandis where ONA content changes are associated with different host plants. ONA change occurs during development. Male fire ants are haploid during early development, but double their ONA before maturity. Boll weevils are largely 2N throughout their life, although in 1/4 to 3/4 of their cells may have 15 to 20 percent additional ONA.

Publications: 88/01 to 88/12

JOHNSTON, J.S., ELLISON, J.R. and VINSON, S.B. 1989. Flow cytometric determination of insect ONA as an aid to the description, identification, and control of the imported fire ant. Vinson SB McCOWN JL (eds.) Proc. Imported Fire Ant Sympo.

JOHNSTON, J.S. and ELLISON, J.R. 1989. ONA heterogeniety within and among fire-ants of the genus Solenopsis in southern United States: Evidence from flow cytometry. Submitted to Cytometry.

JOHNSTON, J.S. and ELLISON, J.R. 1988. Triploidy in a polygynous population of the imported fire ant Solenopsis invicta in Walton Co., Georgia. Submitted to Hereditas.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. A method for determination of ONA content of nuclei of insects by flow cytometry. Submitted to Cytometry.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

15.009 CRISO138239 RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN SORGHUM

HART G E; MGAILL C; MULLET J; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6998 Project Type: HATCH Agency IO: CSRS Period: O6 JUN 89 to O5 JUN 92

Objectives: 1) Identify unique-sequence and low-copy-number ONA clones that hybridize to digested Sorghum DNA, 2) identify Sorghum accessions that display RFLPs for many clones, 3) produce a saturated RFLP map of Sorghum, and 4) identify RFLP alleles in agronomically important Sorghum accessions.

Approach: Sorghum accessions that differ in geographical origin, race cytotype, single-gene characters, and agronomically important traits

will be probed with Sorghum gDNA and cONA clones and heterologous maize clones. Clones that produce a strong signal and a simple hybridization pattern on Southern blots will be used to study accessions that display RFLPs at many loci in order to produce a saturated RFLP map of Sorghum. Following this, the allelic constitution at mapped RFLP loci of accessions that differ in agronomically important traits will be determined.

15.010 CRISO136422 CELL WALLS OF MAIZE PERICARP

HOOO E E; Biology; Utah State University, Logan, UTAH 84322. Proj. No.: UTAOO615 Project Type: HATCH Agency IO: CSRS Period: 28 OCT 88 to 30 SEP 91

Objectives: A. To isolate and characterize a c ONA clone for a maize pericarp hydroxyproline-rich cell wall glycoprotein. B. To determine the developmental expression pattern for this gene at the mRNA and protein levels. C. To determine the tissue specific pattern of expression for this gene at the mRNA and protein levels. O. To determine the level of this protein present in several diverse varieties of maize.

Approach: A cONA clone(s) for the maize pericarp cell wall protein (PC-1) will be isolated using an oligonucleotide probe based on protein sequence obtained previously. Appropriate clones will be sequenced to determine relatedness and sequence homology to dicot genes. Selected clones will be used as probes on Northern blots to determine developmental and tissue specific patterns of expression, and on Southern blots to determine gene family size. Antibodies previously raised in rabbits against PC-1 will be used to determine developmental and tissue specific patterns of protein accumulation. These antibodies can also be used on tissue slices or tissue prints to determine cell type localization of the protein. The cONA clone(s) and antibodies described above will be used in similar experiments on several varieties of maize to determine if PC-1 is associated with pericarp thickness and/or toughness. These traits are associated with the pericarp of popcorn and dent corn.

CM 16 RICE

16.001 CRISO130000 THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES

RODRIGUEZ R L; Genetics; University of California, Davis, CALIFORNIA 95616.

Proj. No.: CA-D*-GEN-4770-CG Project Type: CRGD Agency ID: CRGO Period: 15 SEP 86 to 30 SEP 88

Objectives: PROJECT 8600199. The objective of this proposal is to isolate and characterize the alpha-amylase genes of rice using recombinant DNA technology. Characterization of these genes will include; the determination of their amino acid and nucleotide sequences; mapping of the genes to the rice chromosomes; localization of the site(s) of mRNA synthesis using in situ hybridization; construction of gene fusions between the alpha-amylase promoter and the CAT gene.

Approach: The purpose of this research is to develop rice alpha-amylase as a model system for the study of plant gene expression, with the expectation that the resulting information will facilitate future attempts to engineer agronomically important traits into plants.

Progress: 86/09 to 88/09. The objectives of the previous proposal were to isolate and characterize the rice alpha-amylase gene family. Toward this end, rice cDNA and genomic libraries were constructed and probed with various heterologous probes. These libraries yielded two cDNA and 28 genomic clones for rice alpha-amylase. Using DNA/DNA hybridization analysis, the 28 genomic clones were assigned to five groups and two of these groups were found to correspond to the two cDNA clones. Nucleotide sequence analysis of the two cDNA clones and two of the genomic clones have been completed. Except for sequencing the remaining members of the family and assigning isozymes to cloned genes, the characterization of the rice alpha-amylase gene family is nearly complete.

Publications: 86/09 to 88/09
SIMMONS, C. R. and RODRIGUEZ, R. L. (1988).
High level expression of rice alpha-amylase from seed and callus tissue. In: Enzymology of Agricultural Enzymes, P. Sonnett and J. Whitaker, eds., ACS Books, New York. In Press.

16.002 CRISO131311 HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE

LEACH J E; Plant Pathology; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KANO0667 Project Type: HATCH
Agency ID: CSRS Period: O1 JUL 87 to 30 SEP 90

Objectives: To analyze the genome of the plant pathogenic bacterium, Xanthomonas campestris pv. oryzae (hereafter, XCo), using restriction fragment length polymorphism (RFLP) mapping. To study the genes governing race specificity in XCo. To examine the role of bacterial attachment in the race-specific induction of resistance.

Approach: To approach these objectives, we will use restriction fragment length polymorphism analysis combined with Southern blot hybridizations. In addition, we will use the technologies of recombinant DNA and recombinational genetics combined with transposon mutagenesis.

Progress: 88/01 to 88/12. The effects of mixed inoculations (using isolates of two different Xanthomonas campestris pv. oryzae (Xco) races, one compatible (C) and one incompatible (I)) on bacterial multiplication and the disease reaction in rice were measured. Leaves of cultivars Cas 209 and IR1545-339 were inoculated with the following mixtures of Xco: 1 compatible (race 1,) : 1 \bar{i} ncompatible (race 2, I), 1 C : 10 I, 10 C : 1 I, 1 C : 100 I, and 100 C : 1 I. Growth rates of isolates in mixed inoculations were compared with those in individual inoculations (race 1 or 2 alone). When leaves were inoculated with 1:1 mixtures, the growth rate of race 2 (incompatible) isolates was severely restricted compared to that of the control (race 2 alone). The same effects were observed in a host in which all races were compatible indicating an interaction between the bacterial isolates was effecting the growth of the race 2 isolate. When leaves were inoculated with mixtures of 10 I : 1 C or 100 I : 1 C, the growth rates of both isolates were the same until the race 2 isolate reached 10 -10 cfu/leaf. Then, the growth rates of both isolates slowed. The results of these experiments support the hypothesis than once induced, resistance is phenotypically dominant over susceptibility.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

16.003 CRISO141190 ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS

IN CEREALS

SCHAEFFER G W; Beltsville Agr Res Center,
Beltsville, MARYLAND 20705.

Objectives: Identify, isolate, clone and characterize genes fine structure and functionassociated with elevated lysine levels for plants regenerated from cells resistant to aminoethylcysteine & cells insensitive to inhibitory levels oflysine plus threonine. The first enzyme to be studied in detail is diaminopimelate dehydrogenase whose presence has been demonstrated in rice.

Approach: The structure and function of the gene(s) along the lysine pathway in cereals will be characterized by: a) profiling electrophoretically the seed storage proteins of mutants & the amino acid composition of 30-50 proteins established; b) polyadenylated RNA of variants will be translated in vitro & early polypeptide products separated two dimensionally to establish direct relationships between phenotypes & protein

composition, cDNA clones & probes for those clones will be created from amino acid sequences & other methods for the study of gene regulation & temporal expression; c) the fine structure & functional promoters of genes will be defined & sequenced. Additionally work will be extended to determine whether insta-bility induced by tissue culture may be due to the mobilization of DNA elements and to improve plant regeneration from callus and cell suspensions. Beltsville, MD; Rm 127, Bg.O11A; BL-2; 12/85; Scientists & technicians: G. Schaeffer; L.Wenko; F.Sharpe, Jr.; J.Dudley; L.Weaver; L.Baustiloos.

Progress: 88/01 to 88/12. The research has focused on the continued amino acid analyses of single seeds to identify heritable high lysine lines from selfed lines, crosses with original parents and backcrosses to high lysine mutants. Fifth generation seeds are now available and are being prepared for field tests. Tissue culture cell lines of high lysine plants have been established and are being utilized for biochemical isolation of specific proteins and characterization of the lines. Proteins of rice endosperm mutants have been fractionated into solubility classes and the amino acid characteristics determined. The major increase in lysine occurs in the salt soluble globulin fraction. Not only is there a shift in the quantity of individual proteins in the mutant but some types of protein appear to be specifically modified as well in the mutant. Currently unique proteins are being isolated, monitored with 3H-lysine, and will be purified in the weeks ahead. Specific genes will be isolated from these mutant lines. This research will lead to new basic information on the synthesis of lysine in rice and the release of new rice germplasm.

Publications: 88/01 to 88/12

CHOWDHURY, M.K.U., SCHAEFFER, G.W., SMITH, R.L. and MATTHEWS, B.F. 1988. Mole- c ular analysis of organelle DNA of diffe rent subspecies of rice and the genomic stability of mtDNA in tissue cultures of rice. Theor. Appl. Genet.76:533-539.

SESEK, S., BOROJEVIC, K. and SCHAEFFER, G.W. 1988. In vitro production of dihpaloids via anther culture in wheat. 7th Internatl. Wheat Genetics Sym- posium, Cambridge University, England, July 13-19, 1988. (Abstract).

SCHAEFFER, G.W. 1988. Segregation for endosperm lysine and protein as well as infertility from crosses of in vitro selected rice. J. Cellular Biochemistry, P roc. 17th Ann. Mtg. UCLA Symp. on Mol. & Cell. Biol., p. 203. (Abstract).

SCHAEFFER, G. 1988. Segregation for endosperm lysine and p rotein as well as infertility from crosses of in vitro selected rice. Proceedings of 22nd Rice Technical Working Group, U. of California, Davis, June 1988. (Abstract).

SCHAEFFER, G.W. 1988. Segregation for increased lysine from crosses of in vitro selected mutants of rice. Proceedings o f 6th Congress of Federation of Euro- pean Societies of Plant Physiology, Split, Yugoslavia, Sept. 1988. (Abstract).

SCHAEFFER, G.W. 1988. Role of microspores and anther culture in advancing technologies. In: Advances in Cell Culture, edited by K. Maramorosch and G. H.

Sato. (Book chapter). Academic Press, N.Y.

16.004 CRISO141166 MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES

MATTHEWS B F; Agricultural Research Service; Beltsville Agr Res Center, Beltsville, MARYLAND 20705.

Proj. No.: 1275-22000-003-00D

Project Type: INHOUSE

Agency ID: ARS Period: 01 APR 86 to 25 JUN 87

Objectives: Develop technologies for bypassing traditional sexual breeding barriers andmaternal inheritance to create variation and unique gene combinations for improving productivity of economically important crop plants, such as wheat, rice and soybean and to map and study the regulation of important genes in these crop systems.

Approach: 1) Transfer portions of chloroplast, mitochondrial and nuclear genomes between species, 2) develop a map and determine the structural organization of mitochondrial genome in the recipient parent and hybrid cell lines, 3) determine the effects of chloroplast transfer on nuclear-chloroplast interaction by monitoring production & regulation of key enzymes (aspartokinase, homoserine dehydrogenases and dihydrodipicolinic acid synthase) involved in synthesis of essential and nutritionally important amino acids, lysine, threonine and methionine from aspartate, and 4) clone nuclear genes encoding these enzymes. Enzyme activities, located mainly inthe chloroplast, will be measured and characterized. Thus, these enzymes will be examined at the gene, mRNA and protein levels to understand mecha- nisms regulating this typical plant biosynthetic pathway. BELTSVILLE, MD; BG 010, RM 9 & 10; BL-1; 12/05/85. B. Matthew, C. Cohen, L. DeBonte.

Progress: 87/01 to 87/06. The cox II gene from the mitochondrion of carrot has been isolated and mapped. A complex intron is present. Portions of the cox II gene and intron have been sequenced to confirm these observations. Research benefited other scientists conducting research in genetic engineering.

Publications: 87/01 to 87/06 NO PUBLICATIONS REPORTED THIS PERIOD.

CRISO049444 GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS

SCHAEFFER G W; CRISS; MATHEWS; Agricultural Research Service; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705. Proj. No.: 1209-20173-003-00D

Project Type: INHOUSE

Period: 01 OCT 84 to 12 AUG 86 Agency ID: ARS

Objectives: Terminate 1209-20173-002, accession 0043396. Start 1209-20173-003 with \$300,000 net to bench funds planned for 1209-20173-002 and \$50,000 from \$4.1 million administrative reduction funds. G. Still is NPS contact. Table 1 - 11F.

Approach: Twelve on-going projects will receive additional funding to accelerate research on genetic engineering, gene mapping and transfer, hormonal regulation, membrane structure, and other biotechnologies for improved cro p productivity, including control of insects, diseases, and other pests. This high-technology research will improve the fundamental understanding of important agricultural problems and lead to innovative solutions to agricultural problems.

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Progress: 84/01 to 84/12. The 160-kilobase chloroplast (ct) DNA of Daucus carota was cloned, and a physical map was constructed. The circular ctDNA map delineates the positions of an inverted repeated region and genes encoding ribosomal RNAs, the large subunit of ribulose biphosphate carboxylase and the 32-kilodalton protein. The ctDNA of a wild species, D. pusillus was also mapped. Although it did show a great deal of variation in restriction endonuclease digestion patterns, the gene positions were found to be conserved. The D. carota 450-kilobase mitochondrial genome was cloned in a bacteriophage lambda vector, and portions of this gene library were partially mapped to the complete circular genome. The ribosomal RNAs and the gene encoding the protontranslocating subunit of the mitochondrial ATPase were localized and mapped. Analysis of flanking regions reveals that these genes are found in only one copy per mitochondrial genome. Changes in gene expression in develoing somatic embryos of D. carota were detected by two-dimensional gel electrophoresis of nascent proteins. Some changes were observed as early as one day following induction of the in vitro developmental sequence by removal of auxin from the culture medium. Putative mutants of D. carota, temperature-sensitive for somatic embryo development, were isolated by a filtration enrichment protocol.

Publications: 84/01 to 84/12
DE BONTE, L.R., MATTHEWS, B.F., and WILSON,
K.G. 1984. Variation in plastid and

mitochondrial DNAs in the genus Daucus. Amer. J. Bot. 7:932-940.

MATTHEWS, B.F., DE BONTE, L.R. 1985.
Chloroplast and mitochondrial DNAs of the carrot and its wild relatives. Plant Molec. Biol. Reporter. (In press).

MATTHEWS, B.F. and WIDHOLM, J.M. 1985.
Organelle DNA compositions and isoenzyme expression in an interspecific hybrid of Daucus. Molec. Gen. Genet. (In press).

DIENER, T.O., OWENS, R.A., and CRESS, D.E. 1984. Plant viroids: new diagnostic methods...agriculture. In: Control of Virus Diseases, E. Kurstak and R.G.

Marusyk, eds., Dekker, New York, pp. 345-360. OWENS, R.A., KIEFER, M.C., and CRESS, D.E. 1985. Biological activity of cloned ...cDNAs. In: Subviral Pathogens of Plants

...cDNAs. In: Subviral Pathogens of Plants and Animals, Maramorosch and McKelvey,

eds., Academic Press, NY. (In press).
HAMMOND, R.W., KIEFER, M.C., CRESS, D.E. and
OWENS, R.A. 1984. Probing viroid
structure-function...cDNAs. In: Molec. Form
and Function of Plant Genome, Plenum, NY.
(In press).

16.006 CRISO131478 CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE

WU R; Biochemistry Molecular & Cell Biology; Cornell University, Ithaca, **NEW YORK** 14853. Proj. No.: NYC-181306 Project Type: STATE Agency ID: SAES Period: 01 JUL 87 to 30 SEP 90

Objectives: Our goal is to increase the fundamental knowledge on the molecular and cell biology of rice by first cloning and sequencing several important genes. We plan next to isolate middle repetitive sequences and develop suitable vectors in order to insert desirable genes into rice chromosomes.

Approach: Our research plan involves the isolation and characterization of rice genes; isolation of mutants in rice; construction of factors for promoting stable integration of specific genes into the rice genome; and transferring genes into the rice genome for expression, and to regenerate rice plants.

16.007 CRISO132819 ISOLATION AND ANALYSIS OF GENES IN RICE

WU R; Biochemistry Molecular & Cell Biology; Cornell University, Ithaca, **NEW YORK** 14853. Proj. No.: NYC-181406 Project Type: HATCH Agency ID: CSRS Period: 01 SEP 87 to 30 SEP 90

Objectives: Our objectives include the following aspects in studying the chloroplast genes in rice: cloning and DNA sequence analysis of several important genes which code for proteins essential for photosynthesis, CO(2) fixation, etc., analyzing the expression of these chloroplast genes, analyzing the process of recombination.

Approach: Our approach will be: isolate rice chloroplast DNA and construct gene libraries in plasmic vectors. Identify specific genes of interest and determine the DNA sequence. Isolate mRNA and carry out hybridization analysis, including the mapping of the 5' and 3' ends of the mRNA corresponding to the gene of interest. Analyze the pattern of recombination by mapping the location of the pseudogenes or transposed DNA segments. Determine the nucleotide sequence at the junction of the transposed DNA segments and look for clues of target sequences for recombination.

16.008* CRISO132023
BIOCHEMICAL GENETICS OF HOST-PATHOGEN
INTERACTIONS

MAGILL C; Plant Pathology & Microbiology; Texas A&M University, College Station, **TEXAS** 77843

Proj. No.: TEXO6890 Project Type: HATCH Agency ID: CSRS Period: 19 JUN 87 to 31 MAY 92

Objectives: THE LDNG TERM GDAL DF THIS PROJECT IS TO UNDERSTAND THE GENETIC BASIS FOR MDLECULAR EVENTS THAT DCCUR IN THE INTERACTION BETWEEN PLANTS AND PDTENTIAL PATHDGENS THAT LEAD EITHER TO DISEASE DR RESISTANCE. ULTIMATELY THE ABILITY TO IDENTIFY AND CLONE GENES INVOLVED IN HOST RESISTANCE DR TOLERANCE WILL PERMIT THE USE DF GENETIC ENGINEERING TECHNOLOGY TO PRODUCE IMPROVED VARIETIES. SPECIFIC DBJECTIVES ARE: 1) TD DEMDNSTRATE GENE ACTIVATION IN CDRN AND RICE EXPOSED TD FUNGAL PATHDGENS, 2) TD CLDNE GENES WHICH ARE INVOLVED IN HOST DEFENSE, 3) TD DETERMINE IF PHENYLPROPANDID SYNTHESIS PATHWAYS PLAY A ROLE IN RESISTANCE DR TDLERANCE IN CEREALS, 4) TD DETERMINE IF DEFENSE RESPONSE GENES ACCOUNT FOR SINGLE GENE RESISTANCE, MULTIGENIC TDLERANCE, DR HYPERSENSITIVE RESPONSE.

Approach: THE TIMING AND LEVELS OF NEW PROTEINS SYNTHESIZED FOLLDWING INDCULATION OF CORN WITH RUST AND RICE WITH BLAST WILL BE ESTABLISHED DN 2 D GELS. MESSENGER RNA ISDLATED FROM CDMPATIBLE AND INCOMPATIBLE INTERACTIONS WILL BE USED TD MAKE CONA LIBRARIES. DIFFERENTIAL HYBRIDIZATION WILL BE USED TO SCREEN EACH LIBRARY FOR UNIQUE MRNAS; THUS IDENTIFYING GENES THAT MAY FUNCTION IN VIRULENCE, RESISTANCE, DR TOLERANCE. ENZYME ACTIVITY AND NDRTHERN BLDTS WILL BE USED TO IDENTIFY AND QUANTITATE EXPRESSION OF PHENYLPROPANOID PATHWAY GENES, AND SOUTHERN HYBRIDIZATION WILL BE USED TO DETERMINE THE HOST OR PATHOGEN DRIGIN OF SPECIFIC CONAS.

Progress: 88/01 to 88/12. Time-course studies of the interaction between maize and Puccinia polysora over the first 36 hours of infection do not reveal any differences at the microscopic level between B37R, a resistant line, and the near-isogenic susceptible host, B37. Though new proteins are produced in both lines within 14 hours of inoculation, we have not been able to identify the proteins or enzymes involved. Df several PAL pathway enzymes examined for total activity changes or isozymes, only peroxidase shows differences in the appearance of isozyme bands, but not until later than 36 hours. Auxotrophic mutants have been isolated in three strains of Magnaporthe grisea (Pyricularia oryzae), the causal organism of rice blast. Two of the lines are sister strains with allelic differences for at least four linked loci as identified by host-differential tests. In addition, one is stable and the other is not. The third strain is an isolate from Guyana which is heterokaryon-compatible with the others, and was chosen to provide a potential source of variation at the DNA level. Several probes from other organisms have been tested for use as homologous probes, and the tryp-1 probe from Neurospora has revealed a restriction fragment polymorphism. The six chromosomes of P. oryzae

have been separated by transverse alternating field electrophoresis, and the tryp-1 chromosome identified.

Publications: 88/01 to 88/12
LIVDRE, A.B., GRUBB, P., MAGILL, C.W. and MAGILL, J.M. 1988. Sephadex column procedure for DNA isolation is also useful for detecting dsRNA. Nucl. Acids Res. 16:776.
MAGILL, J.M. and MAGILL, C.W. 1988. DNA Methylation in Fungi. Developmental

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Genetics. (In press.)
LIVDRE, A.B. 1988. Characterization of chloroplast DNA in androgenic albino rice plants. Ph. D. Dissertation. TAMU, College Station. 77p.

16.009 CRISO087248
REGULATION OF GENE EXPRESSION DURING ENDOSPERM
DEVELOPMENT

DKITA T W: Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO0590 Project Type: HATCH Agency ID: CSRS Period: O1 DCT 87 to 30 SEP 90

Objectives: Determine the pathway(s) through which protein bodies are formed during wheat endosperm development. Identify the molecular signals responsible for storage protein sorting and packaging within the cell. Determine the biochemical basis for the differential recruitment of rice glutelin and prolamine mRNA transcripts into translational complexes. Elucidate the gene structure of endosperm specific ADPglucose pyrophosphorylase.

Approach: Protein body formation during wheat endosperm development will be evaluated by electron microscopy and immunocytochemical techniques using gliadin and glutelin antibodies. For protein sorting studies, DNA constructs containing the coding segment of the gliadin and glutelin storage proteins fused to a reporter gene will be transferred into tobacco and the cellular site(s) of accumulation evaluated in different tissues. To identify peptides responsible for intracellular transport, successive 3'-end deletions of the storage protein coding segment will be performed, fused to a reporter gene and transferred into tobacco. Rough endoplasmic reticulum fractions enriched for glutelin and prolamine synthesis will be attempted using differential and sucrose density gradient centrifugation.

Progress: 88/01 to 88/12. Gel retardation studies have shown that nuclear proteins from developing wheat endosperm bind specifically to a gliadin promoter fragment, -113 bp to -231 bp. This fragment contains both a CCAAT box and CACA motif conserved among several cereal seed protein genes. By isolating the appropriate restriction DNA fragments, specific binding activity to both sequence motifs with wheat endosperm extracts has now been demonstrated. In transient expression assays, deletion of this DNA segment results in substantial reduction in gliadin promoter activity. Hence,

the cumulative information from both the transient expression of the gliadin promoter and gel retardation studies indicates that the CACA and/or nearby DNA sequences are important for transcription and the gliadin genes. Immunocytochemical-electron microscopy studies showed that, unlike the prolamines of maize, rice and sorghum, the prolamines of wheat are packaged via the Golgi complex. In many instances, small electron dense vesicles containing proteinaceous material reacted to gliadin antibodies can be observed directly connected to the distal ends of the Golgi cisternae. The structure of the rice prolamine polypeptide was elucidated by analysis of recombinant DNA clones. Unlike all other cereal prolamines analyzed to date, the rice prolamine gene and encoded protein are devoid of repeating DNA and peptide segments, respectively.

Publications: 88/01 to 88/12
KIM, W.T. and OKITA, T.W. 1988. Nucleotide and Primary Sequence of a Major Rice Prolamine. FEBS Lett. 231:308-310.
KIM, W.T., KRISHNAN, H.B., FRANCESCHI, V. and OKITA, T.W. 1988. Formation of Wheat Protein Bodies: Involvement of the Golgi Apparatus in Gliadin Transport. Planta 176:173-182.
KIM, W.T. and OKITA, T.W. 1988. Structure, Expression and Heterogeneity of the Rice

16.010 CRISO143388
CLONING CULTIVAR SPECIFICITY GENES FROM
MAGNAPORTHE GRISEA

Seed Prolamines. Plant Physiol 88:649-655.

DURBIN R D; LEONG S; MAXWELL D; Plant Pathology; University of Wisconsin, Madison, WISCONSIN 53706.

Proj. No.: 3655-22240-001-025

Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 30 SEP 88 to 29 SEP 89

Objectives: Make a molecular and genetic analysis of mechanisms controlling pathogen virulence and variability in the rice blast fungus, Magnaporthe grisea.

Approach: Clone virulence genes by chromosome walking. Construct a genetic marker map of the fungus genome.

Progress: 88/01 to 88/12. Preliminary evidence for transformation of Magnaporthe grisea was obtained using hygromycin as a selective agent.

Publications: 88/01 to 88/12

SKINNER, D.Z., LEUNG, H. and LEONG, S.A.

1988. Molecular mapping and electrophoretic
karyotyping of Magnaporthe grisea. J. Cell
Biochem. Suppl. 12C p. 291. Abstract.

SKINNER, D., LEUNG, H. and Leong, S.A. 1988.

Molecular mapping and electro- phoretic
karyotyping of Magnaporthe grisea (Abstr).

5th Intl Congress of Plant Pathology,
Kyoto, Japan. Accepted August 8, 1988.

SKINNER, D.Z., LEUNG, H. and LEONG, S.A.

1988. Meiotic instability of chromosomal
segments in Magnaporthe grisea.

Phytopathol. Accepted October 3, 1988. Abstract.

17.001 CRISO034309 TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING

DVORAK J; Agronomy & Range Science; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-ARS-4691-CG Project Type: CRGD Agency ID: CRGD Period: O1 SEP 84 to 31 AUG 88

Objectives: Proj 8400544. Dbjective of the project is to recombine wheat chromosomes with the chromosomes of Elytrigia elongata and E. pontica that control high tolerance to salinity. These recombined chromosomes will ultimately be used to develop salt-tolerant wheat.

Approach: High salt tolerance of wild grasses E. elongata and E. pontica is determined by genes on several chromosomes. To find which Elytrigia Chromosome arms carry these genes, we will construct wheat x E. elongata amphiploid lines lacking individually each of the 14 chromosome arms of the Elytrigia genome. Their salt tolerance will be determined in hydroponic cultures and compared to the euploid amphiploid. Those Elytrigia chromosome arms that will appear to carry the genes will be recombined with wheat chromosomes by induced homoeologous recombination.

Progress: 88/01 to 88/12. The genetic control of salt tolerance in Lophopyrum elongatum was investigated in the amphiploid from cross between Chinese Spring wheat and L. elongatum. The amphiploid was more salt tolerant than wheat when grown in a solution culture containing 250 mM NaCl in the greenhouse. A set of 21 substitution lines with the chromosomes of L. elongatum individually replacing wheat homoeologues was tested for salt tolerance. Substitution lines containing L. elongatum chromosomes 3E, 4E, and 7E were found to increase salt tolerance.

Publications: 88/01 to 88/12
DVDRAK, J., M. EDGE, and K. RDSS. (1988). Dn
 the evolution of the adaptation of
 Lophopyrum elongatum to growth in saline
 environments. P.N.A.S. 85:3805-3809.

17.002 CRISO135657 GENETICS OF SALT TOLERANCE OF LOPHOPYRUM ELONGATUM AND ITS TRANSFER INTO WHEAT

DVDRAK J; Agronomy & Range Science; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-ARS-4993-CG Project Type: CRGD Agency ID: CRGD Period: 01 JUL 88 to 30 JUN 91

Objectives: PRDJ. 8800591. The objective is to recombine chromosomes 2E, 3E, 4E, and 7E of Lophopyrum elongatum with wheat homoeologues and transfer the salinity tolerance from Lophopyrum into wheat.

Approach: Recombination will be achieved by manipulation of Ph-1 locus of wheat. Recombined chromosomes will be identified by chromosome pairing with telocentrics, by C-banding and hybridization with repeated nucleotide sequences unique to L. elongatum genome.

17.003 CRISOO48899 MOLECULAR CONTROL OF GENE ACTIVITY DURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT

GREENE F C; LITTS J C; KASARDA D D; Agricultural Research Service; Western Regional Res Center, Albany, **CALIFORNIA** 94710. Proj. No.: 5325-20170-006-00D

Project Type: INHOUSE Agency ID: ARS Period: 16 SEP 83 to 31 MAR 86

Objectives: To gain a basic knowledge of the molecular mechanisms that control the expression of major gene systems during reproductive development of the wheat plant.

Approach: Information will be sought on the nature of the control factors that facilitate the massive synthesis of storage proteins during development of the wheat seed, and on the nature of the factors that control the expression of non-storage protein genes in the seed. The experimental approach will involve cloning and isolation of genes that code for gliadins and for selected non-storage proteins, analysis of the coding and control regions of these genes via DNA sequencing, studies of the in vitro transcriptional efficiencies of wheat protein genes, and investigations of the preparation and transcription of hybrid genes that contain both storage and non-storage protein segments.

Progress: 86/01 to 86/03. Construction of cloned cDNA libraries, from mRNAs of two stages of developing wheat seed, has been initiated. Dligonucleotides have been synthesized based on the amino acid and nucleotide sequences of animal glutamate oxaloacetate transaminase, and will be used as probes to isolate the homologous wheat clones. These studies are being continued under CWU 5325-20520-034, Accession No. 0140448.

Publications: 86/01 to 86/03
ND PUBLICATIONS REPORTED THIS PERIOD.

17.004 CRISO044250 EVOLUTION OF POLYPLOID WHEATS VIA AMINO ACID SEQUENCING AND ELECTROPHORETIC STUDIES

KASARDA D D; CALDWELL K A; FULLINGTON J G; Agricultural Research Service; Western Regional Res Center, Albany, **CALIFORNIA** 94710. Proj. No.: 5325-20520-011-00D

Project Type: INHDUSE Agency ID: ARS Period: 12 JAN 78 to 31 MAR 86

Objectives: Determine the species that contributed genomes to polyploid wheats, the changes in these genomes that resulted from polyploid formation or subsequently, and the consequences of thee factors for wheat quality.

Approach: Storage proteins from diploid species related to wheat, especially those of the genera Triticum and Aeuilops, and trom tetraploid and hexaploid wheats will be fractionated into less complex mixtures or individual components for elctropphoretic characterization and amino acid sequencing. High-performance liquid

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chromatography as well as traditional methods of gel filtrationand ion exchange chromatography will be used for protein and peptide purification. Both one-dimensional and two-dimensional methods of electrophoresis will be used. Sequencing will be carried out by automatic and manual techniques. Results will be analyzed in relation to protein structure and gene evolution and the potential for quality improvement or maintenance in wheat through, or in conjunction with, introduction of alien genetic material into the wheat genome..

Progress: 86/01 to 86/03. The minor omega-gliadin component coded on chromosome 1A of bread wheats was purified and characterized by N-terminal amino acid sequencing to demonstrate close relationship of the gene coding for this protein and similar proteins from the species Triticum monococcum. This protein is of special interest in defining gene clustering in wheat because it has been reported to show significant recombination relative to other omega gliadins.

Publications: 86/01 to 86/03 ND PUBLICATIONS REPORTED THIS PERIDD.

17.005 GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS

KASARDA D D; GREENE F C; ANDERSDN D D; Agricultural Research Service; Western Regional Res Center, Albany, **CALIFORNIA** 94710. Proj. No.: 5325-20520-013-00D

Project Type: INHDUSE Agency ID: ARS Period: 12 JAN 78 to 30 APR 86

Objectives: Determination of the structure of the wheat genome and the expression of the genome in protein biosynthesis, leading to control of protein levels and quality in wheat grain.

Approach: The structure of the wheat genome will be explored by use of recombinant DNA techniques to define the structure of the genes coding for wheat storage proteins and to explore the mechanisms by which these genes are expressed. A genomic library will be prepared. Nuclei will be isolated at various stages of endosperm and plant development and gene expression characteristic of these stages will be analyzed. Products of gene transcription and translation, including messenger RNA and the proteins themselves, will be fractionated and characterized by various electrophoretic techniques and other physical methods.

Progress: 86/01 to 86/04. Detailed structural comparisons of gliadin genes are being continued. Dne silent gliadin gene has been detected. Segments of the 5'-flanking regions of gliadin and glutenin genes, which are thought to contain sequences that control gene expression, have been subcloned. A plasmid vector has been prepared for analysis of wheat gene 5'-flanking promoter regions in plant protoplasts. Zea maize and Triticum monococcum suspension cultures have also been obtained for

this purpose. These studies are being continued in CWU 5325-20520-034-00D.

Publications: 86/01 to 86/04

NO PUBLICATIONS REPORTED THIS PERIOD.

17.006 CRISO140448 WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS

GREENE F C; ANDERSDN D D; CAMPBELL D E; Plant Physiology & Chemistry Research Unit; Western Regional Res Center, Albany, **CALIFORNIA** 94710. Proj. No.: 5325-41000-007-00D

Project Type: INHOUSE Agency ID: ARS Period: O1 DCT 85 to 30 SEP 89

Objectives: To identify regions of the wheat genome that control the timing and specific localization of storage protein gene expression in the wheat seed; to determine the molecular basis of such control, and its potential application for improvement of wheat protein quality.

Approach: An interdisciplinary approach involving molecular biology, plant physiology and genetics will be utilized in this research. The structural organization of wheat chromosomal DNA in storage protein genetic loci, including both genes and intergenetic regions, will be determined via DNA hybridization and sequence analysis. Model systems, utilizing transfer of DNA to heterologous and homologous cells, will be developed for the study of wheat storage protein gene expression, and to identify potential regulatory genes. The effects of in vitro DNA sequence modification on theexpression of these genes will be studied at levels of gene transcription/ mRNA synthesis and protein synthesis.

Progress: 88/01 to 88/12. sequencing of the six high-molecular-weight glutenin genes from the wheat cultivar Cheyenne. Continued analysis of the alpha-gliadin wheat storage protein genes and the low-molecular-weight glutenin genes. The LMW studies are continuing in cooperation with University of California, Davis. Completed construction of a large wheat lambda library to be used in specific sequence isolation. Initiated a study of the wheat sucrose synthase genes by isolating two genomic clones homologous to a maize sucrose synthase DNA fragment. Completed an analysis of the optimization of heterologous expression in yeast of a wheat alpha-gliadin gene. Concluded that optimimum expression of the construct was obtained by control of the carbon source and by modifying yeast plasmid constructs carrying the wheat gene to increase alpha-gliadin gene copy numbers. Began the introduction of site-specific changes in storage protein genes for high-level expression in both yeast and E. coli: succeeded in changing sequence sites in a HMW gene to isolate the coding sequence. Initiated tissue culture and plant cell protoplast preparation, and began studying the control of wheat storage protein gene chimeric constructs. Preliminary data suggest positive activating factors control these genes.

Initiated cooperative work with Cornell on transformation and regeration of rice with wheat storage protein gene contructs.

Publications: 88/01 to 88/12

ANDERSDN, D.D., HALFDRD, N.G., FDRDE, J., YIP, R.E., SHEWRY, P.R., and GREENE, F.C. 1988. Structure and analysis of the high-molecular-weight glutenin genes from Triticum aestivum L. cv. Cheyenne. Proc.7th Int.Wheat Gen.Sym.Acc.7/13/88.

GREENE, F.C., ANDERSDN, D.D., YIP, R.E., HALFDRD, N.G., MALPICA-RDMERD, J.-M., and SHEWRY, P.R. 1988. Analysis of possible quality related sequence variations in the 1D glutenin HMW subunit in genes of wheat. Proc.7th.Int.Symp.Acc.7/13/8.

HALFORD, N.G., FDRDE, J., ANDERSDN, D.D., GREENE, F.C., and SHEWRY, P.R. 1988. The structure and expression of genes encoding the HMW subunits of wheat glutenin. Proc. 7th Int. Wheat Genetics Symp. Accepted 7/13/88.

ANDERSDN, O.D., YIP, R.E., HALFDRD, N.G., FDRDE, J., SHEWRY, P.R., MALPICA- RDMERD, J.-M. and GREENE, F. 1988. Nucleotide sequences of 2 HMW glutenin genes from the D-genome of a hexaploid bread wheat. Nucl.Acids Res. Acc. 11/28/88.

ANDERSDN, D.D. and GREENE, F.C. 1988. The characterization and comparative analysis of HMW, glutenin genes from genomes A & B of a hexaploid bread Conclu ded that optimimum expression of the construct was obtained by control of the.

17.007 CRISO049971 CEREAL GRAIN IMPROVEMENT BY RECOMBINANT DNA METHODS

GREENE F C; ANDERSDN D D; USDA-ARS-wrrc Food Proteins Research; 800 Buchanan Street, Albany, CALIFORNIA 94710.

Proj. No.: 8016-21020-001-01X

Project Type: GRANT

Agency ID: ARS Period: O5 DCT 84 to O4 APR 89

Objectives: Determination of the structure and expression of the wheat genome in relation to protein biosynthesis with the objective of improvement of protein quality in wheat watershed agroecosystems to examine the effects of management and physiographic variability on watershed response.

Approach: The structure of the wheat genome will be explored by use of recombinant DNA techniques to define the structure of the genes coding for wheat storage proteins and to explore the mechanisms by which these genes are expressed. Wheat messenger RNA coding for storage protein components will be prepared and complementary DNA will be cloned. Wheat genomic DNA including genes for wheat proteins will be prepared and cloned. Genomic DNAcorresponding to genes being actively transcribed will be prepared and studied by means of cDNA and other DNA probes.

Progress: 88/01 to 88/12. Cooperators in Madrid continued sequencing the first wheat gamma- gliadin gene isolated in this project,

and continued characterization of additional clones. At Albany we screened original and newer lambda genomic libraries and isolated at least 10-15 more genes, representing most members of this gene family from the bread wheat when cultivar Cheyenne. Restriction mapping analysis and subcloning of new isolates are continuing.

Publications: 88/01 to 88/12 ND PUBLICATIONS REPORTED THIS PERIOD.

17.008 CRISO143897 EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO

HDFFMAN D L; BDCKELMAN H E; Agricultural Research Service, Aberdeen, **IDAHO** 83210.

Proj. No.: 5366-21000-004-00D

Agency ID: ARS

Project Type: INHDUSE Period: 20 DEC 88 to 19 DEC 93

Objectives: To determine the feasibility of DNA fragment length polymorphism for genetic analysis & germplasm enhancement in oats. To initiate a linkage map of oats using conventional and molecular markers. To begin determining linkages or associations of molecular markers with economic genes in oats. Evaluate wheat germplasm for vesicular arbuscular mycorrhizal dependency. Develop bioassays to detect allelochemicals in wheat germplasm. Coordinate Uniform Eastern and Southern Soft Red Winter Wheat Nurseries.

Approach: A genomic DNA library of oats has been constructed and clones from this library will be evaluated for copy number. Low copy clones will be screened for polymorphism among genetically diverse oat cultivars and related wild species. Linkage or association of polymorphic markers will be assigned to chromosome with a monosomic series. Linkage among markers will be determined using recombinant inbred populations generated from a wide cross in oats. Wheat germplasm will be evaluated for vesicular/arbuscular mycorrhizae dependency in two steps: 1)selection of accessions with rapid and substantial colonization as a preliminary screen and then 2) evaluation for growth response to colonization under low phosphorus conditions. Bioassay techniques to detect allelochmicals in wheat germplasm will be developed by studying the effects of exudates on germination, seedling growth, dry matter production, etc. of various indicator species. IBC Approval Pending.

17.009 CRISO131127 CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS

LIANG G H; Agronomy; Kansas State University,

Manhattan, KANSAS 66506.

Proj. No.: KANOO658 Project Type: HATCH

Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: Determination of the cross barriers between Sorghum bicolor and the 10-chromosome sorghum species (S. versicolor and S. purpureo-sericeum) and exploration of techniques to overcome those barriers; analysis of the genomic homoeology between S. bicolor and S. halepense; comparison of mt-DNA restriction endonuclease patterns of different species; evaluation of the effects of growth regulators on plantlet development via anther culture; an understanding of the genetic mechanisms governing callus induction and plant regeneration; development of interspecific hybrids between wheat (Triticum aestivum) and Elytrigia elongata for drought and heat tolerance.

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Approach: Pollen germination and pollen tube growth will be monitored for wide crosses by using a florescence microscope; interploidy crosses following chromosome doubling and protoplast fusion will be made. Chromosome pairing relations and N-banding patterns of sorghum species will be studied. Endonucleases Ecor I, Hind III, BamH 1 will be used to detect the restriction patterns of different sorghum species following the extraction of mtDNA. Effects of available growth regulators will be compared in a common nutrient-medium background for their ability to induce callus and to generate plants. F(1) and \$(1) populations of alfalfa will be constructed to study the inheritance of plant regeneration.

Progress: 88/01 to 88/12. For direct generation of wheat haploids via anther culture, we found a combination of 2 mg 2,4,5-T and 2 mg kinetin per liter in the background medium 85D12 was the most effective than sixother auxins; cultivar Pavon produced most calli(14%) and most green plantlets (4%) than other cultivars. A 2-step anther culture system established for sorghum (S. bicolor) produced a number of green plants but none was confirmed as haploid; spontaneous chromosome doubling may be one of the mechanisms involved for the occurrence of diploids through anther culture; callus induction and regeneration were successful for inflorescence culture of sorghum; however, it is genotype dependent; genotype Xin-White No. 1 responded well. From cytogenetic analyses of sorghum-Johnson-grass hybrids (2n=30,40,60), it is proposed that cultivated sorghum is a tetraploid species with the genomic formula AAB(1)B(1) and Johnsongrass a segmental auto-allo-octoploid, AAAA B(1)B(1) B(2)B(2). Mitochondrial RFLP patterns (Ecor I digestion) of 10-chromosome sorghum, S. versicolor distinguishly differed from those of S. bicolor and S. halepense; the latter species had very similar RFLP patterns. Mt-RFLP patterns of paired male sterile and fertile lines and isocytoplasmic strains of sorghum (Ecor I, Hind III, and Bam III digestions) were also compared; cytoplasmic male sterile lines differed from their male counterparts consistently. Among the isocytoplasmic strains, KS36A (S. verticilliflorum cytoplasm), KS38A(S.

Publications: 88/01 to 88/12

TANG, H. and LIANG, G.H. 1988. The genomic relationship between cultivated sorghum (Sorghum bicolor (L.) Moench) and Johnsongrass (S. halepense L.) Pers.): a re-evaluation. Theor. Appl. Genet. 76:277-284.

WAN, Y., SORENSEN, E.L. and LIANG, G.H. 1988. Genetic control of in vitro regeneration in alfalfa (Medicago sativa L.). Euphytica 39:3-9.

WEN, F., BARNETT, F.L. and LIANG, G.H. 1988. Somatic pairing and separation of chromosomes in root-tip cells of regeneration sorghum plants. J. Hered. (in press).

LEE, S.H., MUTHUKRISHNAN, S., SORENSEN, E.L. and LIANG, G.H. 1988. Restriction endonuclease analysis of mitochondrial DNA from sorghum with fertile and male-sterile cytoplasms. Theor. Appl. Genet. (in press).

LIANG, G.H., OI, J. and HASSAWI, D. 1988.
Generation systems for haploid production in wheat. IN: Biotechnology in Agriculture and Forestry (ed. YPS Bajaj).
Springer-Verlag, New York, NY. (in press).

TANG, H. 1988. Cytogenetic and molecular analyses of the genomic relationships of Sorghum: sections Sorghum and Parasorghum. Ph.D. Thesis. Kansas State Univ. 64 p. WEN, F. 1988. Anther culture of Sorghum

bicolor (L.) Moench. M.S. Thesis, Kansas State Univ. 47 p.

17.010 CRISO034185 CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT

SEARS R G; GILL B S; Agronomy; Kansas State University, Manhattan, KANSAS 66506.

Proj. No.: KAN05446 Project Type: CRGO Agency ID: CRGO Period: 15 JUL 84 to 31 JUL 88

Objectives: Proj 8400540. Identify the chromosomal location of genes controlling tissue culture response in wheat. Develop substitution lines containing the major genes responsible for rapid in vitro cell growth and high embryogenesis in wheat. Initiate investigations on the impact of the genes in studies involving their a) use in cloning vectors, b) use in basic developmental research, and c) transfer into other nonregenerable genotypes.

Approach: A monosomic set of the wheat cultivar Wichita will be crossed with a highly regenerable wheat line, ND 7532. Two monosomic plants and 50 immature embryos per plant will be sampled for tissue culture response in each of the 21 chromosomes. Cultures will be evaluated five months. Substitution lines will be initiated into Wichita by backcrossing. Tissue culture growth rate studies on the Chinese Spring ditelosomic series will be conducted to support conclusions.

Progress: 84/07 to 88/07. Four experiments were conducted utilizing the available aneuploid stocks (monos, ditelos, nullitetras) of wheat to locate chromosomes containing genes controlling tissue culture response (tcr) in wheat. The first experiment was monosomic

analysis. A series of 21 monosomics were crossed as females to a highly regenerable genotype, ND7532. Two criteria were used to measure tor during 120 days: frequency of immature embryos forming regenerable calli and callus growth rate. Critical monosomics 2B, 2D, and 7B were comparable to control ND7532 by responding with regeneration frequencies between 91.5-93.5% and average callus growth rates of 3.33-3.43 g. Noncritical monosomics responded with regeneration frequencies <80% and average callus growth rates. To further characterize these critical chromosomes, a second experiment was conducted. Monosomic F(1)plants (monosomic x ND7532) from group 2 and group 7 chromosomes were crossed with a low regenerable genotype Vona. Plants carrying the identified critical chromosomes 2B, 2D, and 7B showed the greatest loss in tcr and was comparable to the control Vona, thus, suggesting that the substitution of a low regenerable chromosome was causing this poor respone. A third experiment was conducted using the available group 2 ditelosomic stocks of Chinese Spring. In general, the long arm ditelos were comparable in respone to the control euploid Chinese Spring. This suggests that the long arms of group 2 chromosomes may have major developmental genes for tcr.

Publications: 84/07 to 88/07

KALEIKAU, E.K., SEARS, R.G. and GILL, B.S.

Monosomic analysis of tissue culture
response in wheat (Triticum aestivum L.).
(Submitted Theoret. Appl. Genet.).

KALEIKAU, E.K., SEARS, R.G. and GILL, B.S.
Control of tissuee culture response in
wheat (Triticum aestivum L.). (Submitted
Theoret Appl. Genet.).

17.011 CRISO034256 GLIADIN GENES OF COMMON WHEAT AND ITS ANCESTORS

HEDGCOTH C; Biochemistry; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KAN-05-462 Project Type: CRG0
Agency ID: CRG0 Period: 15 SEP 84 to

Objectives: PROJ 8400546. The aims of the proposed research are to broaden the understanding at the gene level of several aspects of the multigene families of gliadins, the storage proteins of wheat endosperm. The work will encompass studies of DNA sequences of gene regions, chromosomal location, and spatial arrangement of genes for gliadins. Initial studies concern hexaploid or common wheat, and additional studies will seek some information about tetraploid and diploid progenitors of wheat.

Approach: The research uses recombinant DNA techniques to isolate, characterize, and study arrangements and chromosomal locations of gliadin genes. We prepare gliadin cDNA and cloned it into pBR322 Escherichia coli. Data from genomic sequences will be used to design cDNA probes with higher specificity than is obtainable with large cDNA or complete genomic sequences. These probes, and others, will be used to test restriction fragment ladders for gene sequences for DNA from various sources:

aneuploids and ditelocentrics of hexaploid wheat will be used to identify structural gene locations with regard to particular chromosomes and chromosome arms. The probes will be used for in situ hybridization to wheat chromosomes for similar reasons. Probes also will be used to test progenitors of common wheat for data on gene numbers and linkage.

Progress: 84/09 to 86/11. A genomic clone for a gamma gliadin was selected from a wheat genomic library using a cDNA probe identified as encoding a gamma gliadin. The genomic clone (L10-20) was subcloned, characterized, and sequenced. The encoded protein, 302 amino acids, has a 19 residue signal peptide, a gamma gliadin type mature amino terminus, 15 heptapeptide repeats, a region of higher cystine and charged amino acids content and a polyglutamine region followed by the carboxyl terminus region. Typical plant transcriptional signals are located in flanking regions. This project involved the preparation of mRNA from developing wheat endosperm, a cDNA library with characterization of several cDNA clones for gliadins and the use of these clones to probe a wheat genomic library. One genomic clone is described above. Characterization of others is in progress.

Publications: 84/09 to 86/11 NO PUBLICATIONS REPORTED THIS PERIOD.

17.012 CRISO084848 CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN

HEDGCOTH C; Biochemistry; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KANO0247 Project Type: STATE
Agency ID: SAES Period: 01 JUL 87 to 30 JUN 90

Objectives: The research is to broaden the understanding at the gene level of several aspects of the multigene families to gliadins and glutenins, storage proteins of wheat endosperm, and encompasses studies of DNA sequences of gene regions, chromosomal locations, and spatial arrangements of genes for gliadins and glutenins. Effects of altering selected features in these proteins will be explored.

Approach: Recombinant DNA techniques will be used in the research. A cosmid library of wheat nuclear DNA will provide the means to map individual genes for gliadins and glutenins as well as clusters of genes to assess organization and arrangement. Aneuploid lines of wheat will provide the means to assign genes to specific chromosomes and the respective arm on a chromosome. A yeast expression system will be used in conjunction with site-specific mutagenesis to study structural features of gliadins and glutenins.

Progress: 88/01 to 88/12. Work is in progress to assign chromosomal locations for various gliadin and glutenin genes which have been cloned. The clones include genes for alpha/beta gliadins, gamma gliadins, a low molecular weight glutenin subunit, and a high

molecular weight glutenin. In addition, a repetitive fragment of ONA is being characterized for use as a probe of wheat ONA for mapping by use of restriction fragment length polymorphisms.

Publications: 88/01 to 88/12

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SCHEETS, K. and HEDGCOTH, C. 1988. Nucleotide sequence of a gamma gliadin gene: comparisons with other gamma gliadin sequences show the structure of gamma gliadin genes and the general primary structure of gamma gliadins.

PITTS, E.G., RAFALSKI, J.A. and HEOGCOTH, C. 1988. Nucleotide sequence and encoded amino acid sequence of a genomic gene region for a low molecular weight glutenin. Nucleic Acids Res., in press.

17.013 CRISO130015 ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES

HEOGCOTH C; Biochemistry; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KAN09150 Project Type: CRG0
Agency IO: CRG0 Period: O1 SEP 86 to 31 OCT 88

Objectives: Project 8601771. The purpose of the research is to provide a foundation of basic information about the structure, location, and organization of the multigene family of gliadins and glutenins, storage proteins of wheat endosperm. Hexaploid wheat with its three independently maintained genomes provides an unusual biological system for studies at the gene level. Evolutionary divergence of a large number of very similar genes existing on different genomes and on different chromosomes within a genome can be evaluated. The hexaploid nature and the availability of ancestral varieties, as well as the genetic tools of aneuploids, make the wheat system unique and fascinating for studies of characteristics of multigene families.

Approach: Prepare a cosmid bank of wheat ONA. Use the cosmid bank to characterize clusters of gliadin gene regions. Explore the nature of changes occurring in gliadin genes in somaclonal variants.

Progress: 86/09 to 88/10. Using cONA's for gliadins and glutenis as probes, a hexaploid wheat genomic library was searched for ONA sequences encoding gliadins and glutenins. From this library, clones for a gliadin and for a low molecular weight glutenin were obtained. These clones were characterized and the nucleotide sequence of each was determined. The information from these studies has provided descriptions of the arrangement of gene regions for gliadins and glutenins. In addition, the encoded amino acid sequences provide information on the structure of the encoded proteins--information not obtainable by direct sequencing of the proteins because of inherent difficulties in sequencing proteins in these large multigene families.

Publications: 86/09 to 88/10

SCHEETS, K. and HEOGCOTH, C. 1988. Nucleotide sequence of a gamma gliadin gene: comprisons with other gamma gliadin sequences show the structure of gamma gliadin genes and the general primary structure of gamma gliadins. Plant Sci. 57:141

PITTS, E.G., RAFALSKI, J.A. and HEOGCOTH, C. 1988. Nucleotide sequence and encoded amino acid sequence of a genomic gene region for a low molecular weight glutenin. Nucleic Acids Res., in press.

17.014 CRISO137013 GENETICS OF BIOTYPES IN THE HESSIAN FLY (MAYETIOLA DESTRUCTOR)

BLACK W C; Entomology; Kansas State University, Manhattan, KANSAS 66506.

Proj. No.: KANO0766 Project Type: HATCH Agency IO: CSRS Period: O1 JAN 89 to 30 SEP 92

Objectives: Construct a genetic map of the Hessian fly using a allozyme and restriction fragment length polymorphisms. Map the location of loci in the Hessian fly that condition virulence to wheats having resistance genes H3, H5, H6, H7, H8 and H9. Estimate rates of gene flow among local populations and biotypes of the Hessian fly using allozyme markers, ribosomal DNA markers, and mitochondrial ONA markers.

Approach: Isozyme polymorphisms at 12 genetic loci will be used to construct a recombination map of the Hessian fly genome. Restriction fragment length polymorphisms, in situ hybridizations on salivary polytene chromosomes and the unique sex determination mechanism in the fly will be used to locate linkage groups to particular chromosomes. Once a map has been established it will be used to locate the genes which condition virulence to wheat. Isozyme markers and ribosomal and mitochondrial ONA markers will be used to monitor rates of gene flow in field populations and biotypes of the Hessian fly.

17.015 CRISO134519 CYTOGENETIC ANALYSIS OF HOST PLANT RESISTANCE IN COMMON WHEAT

GILL B S; Plant Pathology; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KAN00720 Project Type: HATCH
Agency IO: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: To isolate a complete set of ditelosomic aneuploids in Wichita wheat. To develop disomic substitution (OS) lines of 21 individual chromosomes each from Auburn and Plainsman V wheats into Wichita, to determine chromosome location of useful genes and genetic linkage with molecular markers. To develop synthetic hexaploid (SH) wheats based on reconstituted tetraploid Wichita (AABB) and wild Aegilops squarrosa accessions.

Approach: Selfed seeds from Wichita monosomics will be cytologically analyzed to identify telocentric chromosomes. Single chromosome substitution lines will be made from Auburn and Plainsman V cultivars in Wichita wheat. Wichita reconstituted AABB stock will be crossed with a large number of A. squarrosa accessions to produce synthetic hexaploid wheats. The disomic substitution and synthetic hexaploids will be evaluated for host plant resistance and other useful traits for use in wheat breeding.

Progress: 88/01 to 88/12. The objectives of the proposed research are to develop ditelosomic stocks in Wichita, develop disomic substitution lines of wheat cvs 'Auburn' and 'Plainsman V' into Wichita and develop synthetic wheats based on reconstituted 'AABB' Wichita and Aegilops squarrosa. In the research completed so far, ditelosomic stocks for one or both arms of chromosomes 3A, 4A, 5A, 6A, 7A, 1B, 2B, 5B, 6B and 7B have been identified by banding analysis. The development of substitution lines has been advanced to BC(1). The extraction of reconstituted 'AABB' is at BC(4) stage.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

17.016 CRISO130010 PHYSICAL MAPPING OF THE GENOME OF WHEAT

GILL B S; SCHWENK F W; Plant Pathology; Kansas State University, Manhattan, KANSAS 66506.

Proj. No.: KAN09450 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 86 to 30 SEP 89

Objectives: PROJECT 8600092. Construct cytogenetic linkage maps for wheat chromosomes 2A, 3B, and 1D. Undertake physical mapping of the linked genes against polymorphic N-bands that provide longitudinal cytological markers for chromosome arms 2Aq, 3Bp, 3Bq, and 1Dp. Analyze parity (or disparity) between genetic maps and cytological maps as influenced by the A, B, and D genomes; chromosome size; arm length; N-band (heterochromatin) number, size, and position; and any other as-yet-unspecified factors

Approach: Telocentric mapping, using the test cross method, will be used to determine linkage of teh polymorphic N-bands with the centromeres. Physical mapping will also be done with intact chromosomes. The extent of distortion caused by the telocentric method will then be calculated. The linkage distance of the N-bands from the centromere. Next, several genes (disease resistance; chlorophyll mutant; isozyme) known to be located on chromosomes 2A, 3B, and 1D will be mapped in relation to N-bands. Several alien gene transfers and a 1Dp deletion line will be analyzed by N-banding. This will physically localize these genes and provide a comparison of the linkage maps with the cytological maps.

Progress: 88/01 to 88/12. Physical mapping of the genome of wheat was carried out by use of polymorphic bands, in situ hybridization and deletions. Recombination mapping of polymorphic

bands was accomplished on wheat chromosome 1D and 6A. The data indicate that there is no recombination in about 50% of the proximal region of each arm of the wheat chromosomes. Since polymorphic bands mark the physical length of wheat chromosomes, they provide valuable reference points for the approximate physical location of genetically mapped markers. In addition, a number of resistance and RFLP loci were mapped. In situ hybridization and chromosome banding were used to map several repeated DNA sequences and alien chromatin segments. In the second year of the grant, Japanese scientists demonstrated a phenomenon akin to hybrid dysgenesis caused by so-called gametocidal chromosomes from Aegilops. In cooperation with T. R. Endo, we have taken advantage of this genetic phenomenon to isolate about 80 deletion chromosomes involving 20 wheat chromosomes. We have isolated about 12 deletions in homozygous condition; the rest remain heterozygous.

Publications: 88/01 to 88/12

- GILL, B.S. and SEARS, R.G. 1988. Current status of chromosome analysis in wheat. In: Chromosome Structure and Function (eds. J. P. Gustafson and R.
- Appels), Proc. Stadler Genet. Symp., Univ. Missouri, Plenum Press, New York. pp. 299-321.
- LAPITAN, N.L.V., SEARS, R.G. and GILL, B.S. 1988. Amplification of specific DNA sequences in wheat-rye hybrids regenerated from tissue culture. Theor. Appl. Genet. 75:381-385.
- RAYBURN, A.L. and GILL, B.S. 1988. Repeated DNA sequence in Triticum: Chromosomal mapping and its bearing on the evolution of B and G genomes. Plant Syst. Evol. 159:229-235.

17.017 CRISO134509 DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS

GILL B S; SEARS R G; COX T S; Plant Pathology; Kansas State University, Manhattan, KANSAS 66506.

Proj. No.: KANO0725 Project Type: HATCH Agency ID: CSRS Period: O1 JUL 88 to 30 SEP 92

Objectives: To construct single copy or low copy genomic probes suitable for RFLP mapping in Aegilops squarrosa and wheat. To determine the chromosome and arm location of DNA probes on 21 chromosomes of common wheat. To score segregation of DNA probes in an F(2) population and construct a genetic linkage map of seven D-genome chromosomes of Aegilops squarrosa.

Approach: Genomic DNA will be digested with PstI and 1-2kb pieces of DNA will be lighted to a plasmid vector and cloned in E. coli. The inserts will be nick translated and probed onto genomic DNA of wheat-barley addition lines, nulli-tetrasomic and ditelosomic stocks of Chinese Spring wheat to determine their arm and chromosome location. For genetic linkage mapping, genomic DNAs form an F(2) population between two accessions of Aegilops squarrosa and will be probed with labeled DNA clones. The

data will be analyzed by the maximum liklihood method to construct a linkage map of 7 D-genome chromosomes of Aegilops squarrosa.

Progress: 88/01 to 88/12. restriction fragment length polymorphisms (RFLPs) as genetic markers in bread wheat, Triticum aestivum, and a wild wheat progenitor, Aegilops squarrosa, was investigated. The objectives were 1) to identify RFLP loci, 2) to assign cDNA sequences onto specific chromosomes and chromosome arms, and 3) to determine linkage relationships between RFLP loci. Utilizing barley cDNA clones as probes, a low level of polymorphism was seen in hexaploid cultivated wheats, however accessions of A. squarrosa revealed extensive polymorphism. Wheat-barley alien addition lines were used to assign 18 cDNA sequences onto specific chromosome groups. Ditelosomic and nullisomic-tetrasomic wheat stocks were used to assign cDNA sequences onto specific chromosome arms. The construction of a partial linkage map was accomplished by analyzing a segregating F(2) population between two homozygous accessions of A. squarrosa exhibiting RFLP loci

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

17.018* CRISO095292 GENETICS AND PHYSIOLOGY OF FUSARIA

LESLIE J F; Plant Pathology; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KANO0547 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: Characterize natural populations of Fusarium moniliforme with respect to mating groups, mating types, vegetative incompatibility groups, meiotic mutants and DNA restriction fragment length polymorphisms, and measure correlations of these traits with pathogenicity. Define the overall regulation of nitrogen catabolism in Fusarium roseum 'Graminearum' and test both naturally occurring variants and mutants induced in the laboratory for nitrogen catabolic activity, pathogenicity and the production of mycotoxins.

Approach: F. moniliforme isolates from corn and sorghum will be subdivided on the basis of their race, mating-type and vegetative incompatibility group. Representatives of each class will be tested for pathogenicity in a corn seedling system, and for the presence of meiotic mutants and DNA restriction fragment length polymorphisms. Nitrogen catabolic activity in mutant and wild-type strains of F. roseum 'Graminearum' will be measured by dry weight of cultures grown in shake flasks, linear growth rates in race tubes, resistance to common nitrogen analogs and defects in enzymes such as nitrate reductase, glutamate dehydrogenase and glutamine synthetase. Mutants defective in nitrogen catabolism will be tested for pathogenicity in a corn seedling system.

Progress: 88/01 to 88/12. We have completed classical genetic studies of nitrate metabolism in F. moniliforme. We mapped all seven nit mutants with respect to each other and tested nitrate reductase levels of these mutants (no activity was detected). nit1 and nit3 mutants were recovered most frequently, but the relative frequencies with which the mutants occurred could be altered by changing the nitrogen source in the minimal chlorate medium. Different strains throw chlorate sectors at different frequencies. These frequency differences are heritable as a quantitative trait and are consistent with a transposable element as the cause for the sectoring phenomenon. A series of five crn mutants (chlorate-resistant, utilize nitrate) were also characterized. All of these mutants had detectable nitrate reductase activity, and two of them appear to be allelic with different nit mutants. The other three loci may be involved in regulation or in nitrate uptake. We have continued to characterize populations of F. moniliforme using vegetative compatibility as our measure of variability. At least 40 different vegetative compatibility groups (VCGs) have now been identified. In general F. moniliforme populations are highly variable with respect to this trait. We have also described a novel phenomenon - termed "heterokaryon self-incompatibility" - which can give false negatives in complementation tests; in our studies this trait is under the control of a single nuclear gene.

Publications: 88/01 to 88/12
KLITTICH, C.J.R. and LESLIE, J.F. 1988.
 Nitrate reduction mutants of Fusarium
 moniliforme (Gibberella fujikuroi).
 Genetics 118:417-423.
KLITTICH, C.J.R., CORRELL, J.C. and LESLIE,
 J.F. 1988 Inheritance of sectoring

J.F. 1988. Inheritance of sectoring frequency in Fusarium moniliforme (Gibberella fujikuroi). Experimental Mycology 12:289-294.

KLITTICH, C.J.R. and LESLIE, J.F. 1988.

Multi-well plates for complementation tests of Fusarium. Fungal Genetics Newsletter 34:21-22.

KLITTICH, C.J.R. and LESLIE, J.F. 1988. Chlorate-resistant, nitrate-utilizing mutants of Fusarium moniliforme (Gibberella fujikuroi). Journal of General Microbiology (in press).

17.019* CRISO141190 ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS

SCHAEFFER G W; Beltsville Agr Res Center, Beltsville, MARYLAND 20705. Proj. No.: 1275-22000-004-00D Project Type: INHOUSE Agency ID: ARS Period: O1 APR 86 to 31 MAR 91

Objectives: Identify, isolate, clone and characterize genes fine structure and functionassociated with elevated lysine levels from plants regenerated from cells resistant to aminoethylcysteine & cells insensitive to inhibitory levels oflysine plus threonine. The

first enzyme to be studied in detail is diaminopimelate dehydrogenase whose presence has been demonstrated in rice.

Approach: The structure and function of the gene(s) along the lysine pathway in cereals will be characterized by: a) profiling electrophoretically the seed storage proteins of mutants & the amino acid composition of 30-50 proteins established; b) polyadenylated RNA of variants will be translated in vitro & early polypeptide products separated two dimensionally to estab-lish direct relationships between phenotypes & protein composition, cDNA clones & probes for those clones will be created from amino acid sequences & other methods for the study of gene regulation & temporal expression; c) the fine structure & functional promoters of genes will be defined & sequenced. Additionally work will be extended to determine whether insta- bility induced by tissue culture may be due to the mobilization of DNA elements and to improve plant regeneration from callus and cell suspensions. Beltsville, MD; Rm 127, Bg.O11A; BL-2; 12/85; Scientists & technicians: G. Schaeffer; L.Wenko; F.Sharpe, Jr.; J.Dudley; L.Weaver; L.Baustiloos.

Progress: 88/01 to 88/12. The research has focused on the continued amino acid analyses of single seeds to identify heritable high lysine lines from selfed lines, crosses with original parents and backcrosses to high lysine mutants. Fifth generation seeds are now available and are being prepared for field tests. Tissue culture cell lines of high lysine plants have been established and are being utilized for biochemical isolation of specific proteins and characterization of the lines. Proteins of rice endosperm mutants have been fractionated into solubility classes and the amino acid characteristics determined. The major increase in lysine occurs in the salt soluble globulin fraction. Not only is there a shift in the quantity of individual proteins in the mutant but some types of protein appear to be specifically modified as well in the mutant. Currently unique proteins are being isolated, monitored with 3H-lysine, and will be purified in the weeks ahead. Specific genes will be isolated from these mutant lines. This research will lead to new basic information on the synthesis of lysine in rice and the release of new rice germplasm.

Publications: 88/01 to 88/12

CHOWOHURY, M.K.U., SCHAEFFER, G.W., SMITH, R.L. and MATTHEWS, B.F. 1988. Mole- c ular analysis of organelle DNA of diffe rent subspecies of rice and the genomic stability of mtDNA in tissue cultures of rice. Theor. Appl. Genet.76:533-539.

SESEK, S., BOROJEVIC, K. and SCHAEFFER, G.W. 1988. In vitro production of dihpaloids via anther culture in wheat. 7th Internatl. Wheat Genetics Sym-posium, Cambridge University, England, July 13-19, 1988. (Abstract).

SCHAEFFER, G.W. 1988. Segregation for endosperm lysine and protein as well as infertility from crosses of in vitro selected rice. J. Cellular Biochemistry, P roc. 17th Ann. Mtg. UCLA Symp. on Mol. & Cell. Biol., p. 203. (Abstract).

SCHAEFFER, G. 1988. Segregation for endosperm lysine and p rotein as well as infertility from crosses of in vitro selected rice. Proceedings of 22nd Rice Technical Working Group, U. of California, Davis, June 1988. (Abstract).

SCHAEFFER, G.W. 1988. Segregation for increased lysine from crosses of in vitro selected mutants of rice. Proceedings o f 6th Congress of Federation of Euro- pean Societies of Plant Physiology, Split, Yugoslavia, Sept. 1988. (Abstract).

SCHAEFFER, G.W. 1988. Role of microspores and anther culture in advancing technologies. In: Advances in Cell Culture, edited by K. Maramorosch and G. H.

Sato. (Book chapter). Academic Press, N.Y.

17.020* CRISO141166 MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES

MATTHEWS B F; Agricultural Research Service; Beltsville Agr Res Center, Beltsville, MARYLAND 20705.

Proj. No.: 1275-22000-003-00D

Project Type: INHOUSE Agency ID: ARS Period: O1 APR 86 to 25 JUN 87

Objectives: Develop technologies for bypassing traditional sexual breeding barriers andmaternal inheritance to create variation and unique gene combinations for improving productivity of economically important crop plants, such as wheat, rice and soybean and to map and study the regulation of important genes in these crop systems.

Approach: 1) Transfer portions of chloroplast, mitochondrial and nuclear genomes between species, 2) develop a map and determine the structural organization of mitochondrial genome in the recipient parent and hybrid cell lines, 3) determine the effects of chloroplast transfer on nuclear-chloroplast interaction by monitoring production & regulation of key enzymes (aspartokinase, homoserine dehydrogenases and dihydrodipicolinic acid synthase) involved in synthesis of essential and nutritionally important amino acids, lysine, threonine and methionine from aspartate, and 4) clone nuclear genes encoding these enzymes. Enzyme activities, located mainly inthe chloroplast, will be measured and characterized. Thus, these enzymes will be examined at the gene, mRNA and protein levels to understand mecha- nisms regulating this typical plant biosynthetic pathway. BELTSVILLE, MD; BG 010, RM 9 & 10; BL-1; 12/05/85. B. Matthew, C. Cohen, L. DeBonte.

Progress: 87/01 to 87/06. The cox II gene from the mitochondrion of carrot has been isolated and mapped. A complex intron is present. Portions of the cox II gene and intron have been sequenced to confirm these observations. Research benefited other scientists conducting research in genetic engineering.

Publications: 87/01 to 87/06 NO PUBLICATIONS REPORTED THIS PERIOD.

17.021 CRISO142353 MOLECULAR GENETIC BASIS OF SPECIFICITY IN CEREAL RUSTS

BUSHNELL W R; ROELFS A P; SCHAFER J F; Agricultural Research Service, St Paul, MINNESOTA 55108

MINNESOTA 55108. Proj. No.: 3640-24000-008-00D

gene-for-gene interactions.

Project Type: INHOUSE Agency ID: ARS Period: O1 OCT 87 to 31 MAR 88

Objectives: To determine the genetic and molecular genetic bases of host-parasite specificity in cereal rusts. The research will provide the genetic information and fungal genotypes needed to isolate race-specific genes for avirulence and virulence toward the ultimate goal of isolating and identifying the gene products that control specificity in

Approach: Genes for avirulence and virulence in one or more rust organisms will be mapped using classical genetic markers, restriction fragment length polymorphisms (RFLP's) and other molecular genetic techniques. Avirulence/ virulence genotypes will be determined on host plants with known genotypes for resistance/susceptibility. Corresponding genes for resistance/suscepti-bility will be analyzed in host lines as necessary. Techniques for isola- tion of DNA from rust fungi will be developed for southern blot analyses. Methods for introduction of DNA into rust fungi will be developed for production of genetically transformed variants. Emphasis will be on leaf and stem rust fungi of wheat. Other rust fungi will be used when necessary to develop new techniques and concepts.

Progress: 88/01 to 88/12. To facilitate genetic studies on Puccinia graminis, experiments on teliospore production and germination were initiated. A diverse group of susceptible seedling and adult wheat plants were infected and examined for teliospore production. Preliminary results indicate that the wide variability seen in teliospore production is inherent in the rust isolates rather than the host. Teliospore germination was found to be induced by heat shock. Incubation of teliospores at 30-32 C for 2 days increased the maximum germination frequency from 1.3% to 19%. Attempts were continued to obtain pathogenic, axenic cultures of Puccinia graminis f. sp. tritici suitable for genetic transformation. Four isolates (from races 32, 36, 126 and an orange mutant of KBCS) were established as permanently growing mycelial cultures, but none were pathogenic when applied to mesophyll of wheat leaves. Race 36 was found to be a better grower than any North American isolate tested previously.

Publications: 88/01 to 88/12 GRIFFEY, C. A. and ROELFS, A. P. 1988. Heat shock induction of teliospore germination in Puccinia graminis. Phytopathology Abstract. Accepted November 14, 1988.

17.022* CRISO049430 GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS

STEINER W M M; Parasite-predator Bio & Ecol Bio Control of Insects Lab; Agricultural Research Service, Columbia, MISSOURI 65211. Proj. No.: 3622-24000-006-00D

Project Type: INHOUSE Agency ID: ARS Period: O1 OCT 84 to 30 SEP 89

Objectives: Genetically map and characterize chromosomal, morphological, DNA, and allozyme variants of selected entomophagous insects and determine their physiological, developmental

determine their physiological, developmental and behavioral significance and their potential for utilization in bioengineering programs to enhance entomophageefficiency.

Approach: Populations of selected entomophages will be assayed using classical gene- tic techniques and restriction enzyme (DNA) technology to establish gene-tically typed isolines. These will be used to establish linkage maps and study genome structure in the context of predator-parasite/prey models. About 80 % of effort will be devoted to the model offered by Heliothis zea and its entomophages with 20% of effort reserved to develop genetic know- ledge of other models. Selected variants will also be biochemically char- acterized and their distributions in natural populations determined. Lab- oratory tests of physiological, developmental and behavior response under stresses posed by pesticides, temperature and desiccation will reveal whichphenotypes can enhance natural population fitness characteristics. Methods of transferring desirable traits will be developed.

Progress: 88/01 to 88/12. Selection for insecticide resistance in females of the parasitoid Microplitis croceipes was initiated to determine how amenable this parasite of Heliothis spp. is for development of insecticide resistant varieties. After 5 generations of inbreeding, two of five lines demonstrated an increase in LD values, going from 0.82 ug fenvalerate (in 0.5 ul acetone topical application) to a 16-fold increase at 13.12 ug with an accompanying increase in number of males being produced and a decline in line fertility. Selection ceased at the 6th round when the lines were lost due to the increased selection pressure. The observed selection response is a typical one seen for braconid wasps, and suggests this is the upper limit to which the M. croceipes genome may respond to insecticide selection. In other studies, three species of nabids were found to segregate for allozyme variation at an esterase locus (EST-1!) and an adenylate kinase locus (ADK-3). These were not in genetic equilibrium and reduction in the numbers of heterozygotes in nature suggests strong population subdivision. These loci may serve a diagnostic function to differentiate closely related nabid

Publications: 88/01 to 88/12
GRASELA, J.J., STEINER, W.W.M. and MARSTON,
N.L. 1988. Genetic differences at two
allozyme loci in midwest populations of

three species of Nabidae. Comp. Biochem. Physiol. 90B:427-431.

STEINER, W.W.M. 1988. Electrophoretic techniques for the genetic study of aphids In, A.K. Minks and P. Harrewijn (Eds.) "Aphids, Their Biology, Natural Enemies and Control", Vol. B, Elsevier Sci. Publ., Amsterdam, pp. 135-143.

17.023* CRISO097028 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY

SANDS D C; BLAKE T K; Plant Pathology; Montana State University, Bozeman, MONTANA 59717.

Proj. No.: MONBOO231 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 90

Objectives: To isolate and characterize genes in plants controlling development, metabolism and resistance to pathogens. To isolate and characterize genes in beneficial and pathogenic microbes controlling biological control of weeds.

Approach: Using wheat, barley and Sclerotinia-genetic systems will be studied using cell culture, mutagenesis, and DNA methods including recombinant libraries of DNA and their analysis with radioactive probes of cDNA. Restriction mapping will also be used in restriction fragment length polymorphism studies of barley. The barley - Xanthomonas host parasite system will be investigated with these methods. Host range reduction of Sclerotinia will be attempted, for applications in biocontrol of weeds.

Progress: 88/01 to 88/12. Several mutants of Sclerotinia sclerotiorum have been obtained by ultraviolet light-8-methoxy-psoralin treatment. These mutants are restricted in host range, or are auxotrophic, or are sclerotialess. In each case they may be of value as weed pathogens because of their limitation in terms of spread beyond the target host. In the past year we have found that protoplasts can be made, and regenerated from this fungus, and that their nuclear number ranges from one to more than ten. Uninucleate protoplasts would be useful for mutation experiments where auxotrophs and other recessive characteristics are desired.

Publications: 88/01 to 88/12
ZIDACK, N. K., FORD, E., HENSON, J. and
SANDS, D. C. 1988. Uninucleate protoplasts
of Sclerotinia sclerotiorum for genetic
manipulation. APS Abstracts 297.

17.024 CRISO033871 GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY RECIPROCAL CHROMOSOME SUBSTITUTIONS

MORRIS M R; ZEMETRA R S; Agronomy; University of Nebraska, Lincoln, **NEBRASKA** 68583.

Proj. No.: NEB-12-130 Project Type: CRG0 Agency ID: CRG0 Period: O1 AUG 82 to 31 JAN 86

Objectives: Proj. No. 8200338. Reciprocal chromosome substitutions between two winter wheatvarieties, Cheyenne and Wichita, will be used to identify chromosomes with genes for cold tolerance, maturity and flour quality. Test pertinent chromosomes statistically for types of gene actions and interactions; test by electrophoresis for kernel proteins if differences are found between the parental varieties.

Approach: Cold-tolerance tests of the reciprocal substitution lines and their parents will be made on field and greenhouse plantings using crown-freezing and electrolytic conductivity. Data on maturity will be collected on a randomized and replicated field planting. Harvested seeds will be used for milling, dough-mixing and electrophoretic tests.

Progress: 82/08 to 86/01. Duplicate reciprocal sets of chromosome substitution lines between the hard red winter wheat cultivars, Cheyenne and Wichita, developed at the University of Nebraska, were used in field and greenhouse experiments to obtain gene-chromosome associations for maturity. flour quality, and traits related to lodging, photosynthesis and yield. Major genes for maturity were located on chromosomes 3A and 3D of Wichita and on 3A, 3D and 6A of Cheyenne. Wichita genes accelerated heading of Cheyenne whereas Cheyenne genes delayed heading of Wichita. Group 1 chromosome substitutions showed glutenin-protein bands (SDS-PAGE electrophoresis) that differentiated the two cultivars. Wichita 1B in Cheyenne had two bands at 47 Kd that were absent in the Cheyenne 1B in Wichita line. Reciprocal differences in mixing time occurred with 1B substitutions. Duplicates of Cheyenne 2D and 7B in Wichita increased mixing time and mixing tolerance, respectively. Chevenne and Wichita differed significantly in two out of nine traits affecting lodging, and in three out of eight traits affecting photosynthesis and yield. Cheyenne 3B and 6A in Wichita each affected eight traits, in most cases increasing the values over those in Wichita. Wichita 3B in Cheyenne increased the values of five traits in the direction of Cheyenne. Reciprocal effects occurred for 1B (internode length below flag leaf), 3B (number of vascular bundles, width of flag leaf, and width of first leaf below flag leaf), and 3D (basal-internode diameter).

Publications: 82/08 to 86/01

ZAARAWI, W.K. 1985. Chromosomal locations of genes for traits associated with lodging and yield in winter wheat using reciprocal substitution lines. M.S.

Thesis, University of Nebraska-Lincoln. 147

ZEMETRA, R.S., MORRIS, R. and SCHMIDT, J.W. 1986. Gene locations for heading date using reciprocal chromosome substitutions in winter wheat. Crop Sci. 25 (In press). Crop Science Soc. of America, Madison, WI.

AL-QAUDHY, W. and MORRIS, R. 1985.
Gene-chromosome associations for
wheat-lodging traits using reciprocal
substitutions. Agron. Abstrs. 1985 Annual
Meetings, p. 479 Am. Soc. Agron., Madison,

WI.

ZEMETRA, R.S. and MORRIS, R. 1984. An unusual growth habit in a winter wheat chromosome substitution line. (Abstr.). Genetics 107(3Pt.2):s 117-118. Genet.

Soc. Am., Baltimore, MD.

ZEMETRA, R.S., MORRIS, R., MATTERN, P.J. and SEIP, L. 1984. Chromosome locations for genes affecting flour quality in winter wheat using reciprocal substitution lines. Agron. Abstrs. 1984 Annual Meetings, p. 96. Am. Soc. Agron.

17.025 CRIS0047013 GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF DURUM AND COMMON

JOPPA L R; WILLIAMS N D; Agricultural Research Service; Agricultural Research Service, Fargo, NORTH DAKOTA 58103.

Proj. No.: 3915-20050-024-00D

Project Type: INHOUSE Period: 19 FEB 81 to 31 MAR 86

Agency ID: ARS

Objectives: Produce aneuploid and other genetic lines to be used in genetic and cytogenetic analysis and in breeding of superior wheats with resistance to pests and/or improved quality and agronomic characteristics.

Approach: Produce D-genome disomic additions, D-genome disomic substitutions, telosomics, and ditelosomics of durum wheat (Triticum turgidum) using standard and innovative methods. Use these lines, and similar lines in hexaploid wheat (T. aestivum) to map genes on chromosomes, to study the interactions of genes on different chromosomes, and to incorporate new genes from other species. Develop innovative breeding methods for the improvement of durum wheat.

Progress: 86/01 to 86/03. The D-genome disomic substitutions of Langdon durum were crossed with a chocolate brown chaffed mutant of Vic durum wheat. The F(2) progeny lines segregated 3 white chaffed to 1 chocolate brown chaffed with the exception of the crosses to the Langdon 7D(7A) and Langdon 7D(7B) line. Analysis of chromosome pairing indicated that the gene had to be present in two doses to be expressed. Chromosome 7D was able to suppress the effect of the gene. The substitution of each of the chromosomes from a high protein Triticum dicoccoides lines into Langdon durum was completed. These lines will be evaluated to deterime which dicoccoides chromosomes have genes for high protein.

Publications: 86/01 to 86/03 NO PUBLICATIONS REPORTED THIS PERIOD.

17 026* CRIS0091503 RECOMBINANT DNA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY

OLESON A E: BERRYHILL D L: KOFOID K D: Biochemistry; North Dakota State University, Fargo, NORTH DAKOTA 58105. Proj. No.: ND01228 Project Type: HATCH Agency ID: CSRS Period: O1 NOV 83 to 30 SEP 88

Objectives: Clone stress response genes from selected plant species and determine the nucleotide sequences of their polypeptide reading frames and flanking regulatory regions. Elucidate the dynamics of expression of stress response genes under various stress conditions by use of recombinant DNA probes. Clone and determine the sequence of nuclear genes for ribosomal RNA from higher plants. Assess mitochondrial diversity in wheat with recombinant DNA techniques. Determine the effects on wheat mitochondrial DNA of growth in tissue culture and regeneration.

Approach: A generalized stress-response plant enzyme (RNase I) will be purified, partially sequenced (Edman method), and a synthetic oligonucleotide probe prepared. Clones from cDNA and genomic libraries will be isolated, sequenced, and used as probes of specific mRNA levels in stressed plants. A previously cloned maize rRNA gene in a lambda vector will be subcloned into plasmid and M13 phage vectors, and sequenced by the dideoxy method. Mitochondrial DNA will be isolated from several species of Triticum and Aegilops and restriction maps prepared. Sequence homologies will be determined by blotting procedures. Restriction maps of mitochondrial DNA from a single species subjected to tissue culture and regeneration will also be compared.

Progress: 83/11 to 88/09. Mitochondrial DNA has been isolated from five wheat cultivars. Each has an AABBDD nucleus, but the cytoplasmic genomes came from Aegilops squarrosa, Haynaldia villosa, T. aestivum, T. timopheevi, and T. turgidum. The DNA preparations were examined with restriction endonucleases BamHI, EcoRI, HindIII, and XhoI. Labeled probes used for this analysis were from T. aestivum or sorghum mitochondrial DNA. The results indicated that Haynaldia villosa was the B genome donor to T. turgidum and T. aestivum. In other work, a lambda clone of the nuclear ribosomal gene region of maize has been subcloned into Escherichia coli plasmid vectors. Shotgun fragments were cloned into a phage vector, and these were sequenced by the dideoxy method. This gene family is present as tandem repeats on the chromosome. The transcript encodes, in a 5' to 3' direction, 17S, 5.8S, and 26S ribosomal RNAs, with internal transcribed spacers 1 and 2 flanking the 5.8S region. Clones containing the 5.8\$ and spacer regions were used as probes for RFLP analysis of corn DNA. These studies indicated that little variation exists in the internal spacer regions of the multiple copies of this gene family. Sequencing of the 26S region was completed. Comparison of the alpha-sarcin domain of this RNA indicated that the 14-base core was the same as that of all other eukaryotes tested. The base flanking the 5'-end of the core is C

in the case of all animals tested, whereas U is present at this position in corn, yeast and rice

Publications: 83/11 to 88/09

MESSING, J.; CARLSDN, J.; HAGEN, G.;

RUBENSTEIN, I.; and DLESDN, A. 1984.

Cloning and sequencing of the ribosomal RNA genes in maize: The 17S region. DNA 3:31-40.

17.027 CRISO096812 TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES

DUYSEN M E; Botany; North Dakota State
University, Fargo, **NORTH DAKOTA** 58105.
Proj. No.: ND01924 Project Type: HATCH
Agency ID: CSRS Period: 01 NDV 85 to 30 SEP 90

Objectives: To evaluate the use of protoplasts derived from anther microspores and immature zygotic embryo cultures to regenerate cereal plants and develop techniques of inserting cytoplasmic based genes into cereals by recombinant DNA techniques and organelle transplantations.

Approach: Protoplasts isolated from microspores or cells of immature embryos will be cultured in the laboratory to regenerate cereal plants. Studies will include optimizing the hormone balance, culture medium additives, and environmental conditions. Chloroplasts will be transplanted into cereal protoplasts and the latter will be regenerated to plants. Attempts will be made to insert desirable cytoplasmic genes into chloroplasts prior to transplantation to cereal protoplasts.

Progress: 87/10 to 88/09. In collaboration with Dr. Peter Westoff (Univ. Dusseldorf, BRD) 15 different cDNA fragments of the LHC gene family have been isolated from sorghum. Each of these fragments express protein products which are antigenic to the LHCII antibody derived from wheat protein. The LHC gene family is nuclear encoded and cytoplasmic translated. Each DNA fragment has been inserted into the Lambda gtll phage and each of the latter has been inserted and cloned in the E coli bacterium. The phage DNA was reisolated from the bacterium and the plant LHC fraction was ligated into the plasmid vector, bluescript. Bluescript was used to transform E coli and the DNA was examined using miniprep techniques. Five different DNA inserts of the LHC gene family, ranging from 300-1000 bp are currently being sequenced using the T7 DNA polymerase system.

Publications: 87/10 to 88/09
DUYSEN, M., J. EIDE and K. MDGEN. 1988. The
 accumulations of LHCmRNA, LHCII protein and
 plastid pigments over greening in the CD3
 wheat mutant. Plant Physiol. (suppl.) 84.

17.028* CRISO133059
FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR
IDENTIFICATION AND CONTROL OF INSECTS

JDHNSDN J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX06916 Project Type: HATCH Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

Objectives: 1) Develop flow cytometry and in particular total nuclear DNA content determination as a cytotaxonomic tool, for the recognition of host races, biotypes, and crytic species of insects. 2) Catalogue the nuclear DNA content of insects of economic interest. 3) Develop multiparameter DNA flow cytometry using dyes which are specific for total DNA, adenine and thymine rich repetitive DNA, and guanine and cytosine rich repetitive DNA. 4) Demonstrate the developmental pattern and mode of inheritance of DNA content variation for both euchromatic (unique) and heterochromatic (repetitive) insect DNA sequences. 5) Develop methodology to sort insect nuclei and chromosomes as an aid to gene cloning in insects.

Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

Progress: 88/01 to 88/12. We have now had one year of work with this unique new methodology for the study of heritable variation in insects. We have measured the DNA content of over 1500 individual fire ants including 3 native and two imported species. In addition, we have measured the DNA content of over 200 honey bees, nearly 300 boll weevils, plus smaller number of individuals from 10 other species. The results have been unexpected and exciting. In particular we find: DNA content is a diagnostic tool that aids in identification of species, populations and types. To date, very significant and predictable DNA content differences have been found between 5 fire ants species, between 4 species of boll weevil, and between the Africanized form of the honey bee, the European honey bee and the F-1 hybrid between the two forms. DNA content variation exists within and among some populations. The most extreme variation we have observed occurs in a fire ant population in Walton Co., Georgia, were 129 of 515 males, females, and workers show 3N or triploid DNA amounts. The remainder are diploid or haploid as expected. The smallest significant variation occurs between populations of the boll weevil Anthonomus grandis where DNA content changes are associated with different host plants. DNA change occurs during development. Male fire ants are haploid during early development, but double their DNA before maturity. Boll weevils are largely 2N throughout their life, although in 1/4 to 3/4 of their cells may have 15 to 20 percent additional DNA.

Publications: 88/01 to 88/12

JOHNSTON, J.S., ELLISON, J.R. and VINSON, S.B. 1989. Flow cytometric determination of insect DNA as an aid to the description, identification, and control of the imported fire ant. Vinson SB McCOWN JL (eds.) Proc. Imported Fire Ant Sympo.

JOHNSTON, J.S. and ELLISON, J.R. 1989. DNA heterogeniety within and among fire-ants of the genus Solenopsis in southern United States: Evidence from flow cytometry. Submitted to Cytometry.

JOHNSTON, J.S. and ELLISON, J.R. 1988.
Triploidy in a polygynous population of the imported fire ant Solenopsis invicta in Walton Co., Georgia. Submitted to Hereditas.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. A method for determination of DNA content of nuclei of insects by flow cytometry. Submitted to Cytometry.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

17.029 CRISO135474 GENETIC STRUCTURE OF SMALL GRAIN PATHOGEN POPULATIONS AND MANAGEMENT OF GENETIC RESISTANCE

MCDONALD B A; Plant Pathology & Microbiology; Texas A&M University, College Station, TEXAS 77843

Proj. No.: TEXO6928 Project Type: HATCH Agency ID: CSRS Period: 11 AUG 88 to 31 JUL 93

Objectives: 1) Monitor presence and assess severity of small grain diseases in South Texas. 2) Develop electrophoretic genetic markers to study the population genetics of fungal pathogens of small grains. 3) Obtain estimates of the distribution of genetic variability in pathogen populations in Texas. 4) Develop and test gene-management strategies that are likely to control small grain diseases. 5) Compare effects of different gene-management strategies on the genetic structure of pathogen populations. 6) Enlarge and diversify the genetic base of small grains in Texas.

Approach: Isozyme and RFLP genetic markers will be developed first for the wheat/wheat leaf rust pathosystem. These electrophoretic markers will be used to quantify genetic variation in pathogen populations in Texas and to measure the response of P. recondita populations to different gene-management strategies. Wheat containing different types and combinations of resistance genes will be deployed in pure stands and in cultivar mixtures in replicated field plots and compared for effectiveness of disease control.

Progress: 88/01 to 88/12. Knowledge of the population genetics of pathogen populations is needed to make more effective use of resistance genes. Restriction fragment length polymorphisms (RFLPs) are being developed as tools for studying the population genetics of small grain pathogen populations. Rapid miniprep DNA extraction protocols were

developed for the wheat pathogens Septoria tritici and Puccinia recondita. Genomic DAN libraries consisting of anonymous 0.5-2.2 kb fragments of S. tritici and P. recondita DNA were cloned into plasmid pGEM4. Though screening for RFLPs did not begin until December, preliminary results are promising; the first two probes tried from the S. tritici library detected RFLPs in S. tritici. P. recondita isolates will be screened shortly. These preliminary results suggest the RFLPs will provide the tools necessary for studying population genetics of pathogen populations.

Publications: 88/01 to 88/12
MCDONALD, B.A., ALLARD, R.W. and WEBSTER,
 R.K. 1988. Response of two-, three-, and
 four-component barley mixtures to a
 variable pathogen population. Crop Sci.
 28:447-452.

17.030 CRISO096560 CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES

HART G E; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843. Proj. No.: TEXO6751 Project Type: HATCH Agency ID: CSRS Period: O7 AUG 85 to 30 AUG 90

Objectives: To determine the chromosomal locations of a large number of homologous gene loci in Triticum aestivum cv. 'Chinese Spring' and in seven diploid Triticeae species, to determine the genetic map positions of a selected group of these loci in Chinese Spring and in three diploid species, and to use the findings obtained to define homoeologous relationships among individual Triticeae chromosomes and chromosome arms.

Approach: Two classes of gene loci will be studied, namely, loci which encode enzymes and loci that hybridize to unique sequence DNA probes. The chromosomal locations of Chinese Spring genes will be determined by study of compensating nullisomic-tetrasomic strains and ditelosomic strains. In diploid species, gene locations will be determined by study of wheat-alien species disomic and ditelosomic chromosome addition lines. Gene-centromere genetic distances will be determined in hexaploid wheat by the telocentric method and map distances between genes in hexaploid wheat and diploid species by Mendelian methods.

Progress: 88/01 to 88/12. The objectives of this project are to identify and determine the chromosomal locations and genetic map positions of homologous unique-sequece genetic loci in species in the tribe Triticeae and to investigate the genetic organization and evolutionary relationships among Triticeae chromosomes and genomes. Genes that encode enzymes have been studied for several years. Loci defined by the study of restriction-fragment-length polymorphisms (RFLPs) are now receiving major emphasis. Thirty-five DNA probes that definitively identify loci and another 30 probes that probably identify loci were isolated this year.

About 100 RFLP loci were localized in chromosomes in Triticum aestivum cv. Chinese Spring. Several allelic variants at RFLP loci were identified. Southern blots containing genomic DNA from wheat-alien species chromosome addition lines were prepared and are now being analyzed to localize RFLP loci in alien chromosomes. Development of a set of wheat-T. searsii dosomic chromosome addition lines was completed and several ditelosomic lines and substitution lines developed. Several genes were localized in chromosomes in Haynaldia villosa.

Publications: 88/01 to 88/12

- BENEDETTELLI, S. and HART, G.E. 1988. Genetic Analysis of Triticeae Shikimate
- Dehydrogenase. Biochem. Genet. 26:287-301. TULEEN, N.A. and HART, G.E. 1988. Isolation and Characterization of Wheat-Elytrigia elongata Chromosome 3E and 5E Addition and Substitution Lines. Genome 30:289-292.
- WHELAN, E.D.P. and HART, G.E. 1988. A Spontaneous Translocation that Transfers Wheat Curl Mite Resistance from Decaploid Agropyron Elongatum to Common Wheat. Genome 30:289-292.
- DEPACE, C., BENEDETTELLI, S., QUALSET, C.O., HART, G.E., SCARASCIA MUGNOZZA, G.T., VELRE, V. and VITTORI, D. 1988. Biochemical Markers in Triticum x Dasypyrum Amphiploids and Derrived Disomic Addition Lines.
- DEVEY, M.E. and HART, G.E. 1988. Chromosomal Localization of RFLP Loci in Hexaploid Wheat. Proceedings of the Banbury Center Conference on Development and Application of Molecular Markers to Problems in Plant Genetics.
- HART, G.E. and GALE, M.D. 1988. Guidelines for Nomenclature of Biochemical/Molecular Loci in Wheat and Related Species. Proc. 7th Int Wheat Genetics Symp. (In press).
- PIETRO, M.E., TULEEN, N.A. and HART, G.E. 1988. Development of Wheat-Triticum searsii Disomic Chromosome Addition Lines. Proc. 7th Int. Wheat Genetics Symp. (In press).

17.031 CRISO099652 CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIDIZE TO UNIQUE-SEQUENCE-DNA CLONES

HART G E; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843. Proj. No.: TEXO6854 Project Type: CRG0 Agency ID: CRG0 Period: O1 AUG 86 to 31 JAN 89

Objectives: To construct a cDNA library of wheat, to determine the chromosomal locations of unique-sequence loci in Triticum aestivum cv. Chinese Spring (CS), to study evolutionary relationships among genetic loci, chromosomes, and genomes of hexaploid wheat, and to initiate studies of RFLPs in wheat varieties and of the chromosomal locations of homologous loci in wheat relatives.

Approach: Genomic DNA of CS aneuploids will be digested, electrophoresed, blotted onto membranes, and hybridized to cDNA clones. Loci will be assigned to chromosomes and arms based on intergenomic variation in RFLs. Several cDNA clones will be used to probe genomic DNAs of

(1) wheat varieties to identify RFLPs and (2) a wheat-alien chromosome addition line series to localize homologous loci in the alien chromosomes.

Progress: 88/01 to 88/12. The objectives of this research are to isolate DNA probes that are capable of identifying RFLP loci in Triticeae species, to determine the chromosomal locations of a large number of RFLP loci in Triticum aestivum cv Chinese Spring (CS) and to initiate genetic mapping studies of these loci and of the chromosomal locations of homologous loci in wheat relatives. The objectives have by and large been accomplished. Thirty-five probes that definitively identify Triticeae RFLP loci and another 30 probes that probably identify loci were isolated this year. About 100 RFLP loci were localized in chromosome arms in CS. A search was initiated for allelic variation at RFLP loci in 25 wheat varieties of diverse geographical origin and several variants were identified. Southern blots containing genomic DNA from wheat-alien species chromosomes addition lines were prepared and are Now being analyzed in pursuit of the last objective.

Publications: 88/01 to 88/12

- DEVEY, M.E. and HART, G.E. 1988. Chromosomal Localization of RFLP Loci in Hexaploid Wheat. Proceedings of the Banbury Center Conference on Development and Application of Molecular Markers to Problems in Plant Genetics.
- DEVEY, M. and HART, G.E. 1988. Intergenomic Restriction Fragment Length Polymorphisms in Hexaploid Wheat. Agronomy Abstracts, p. 79.

17.032 CRISO136231 CHROMOSOMAL LOCALIZATION AND INTRACHROMOSOMAL MAPPING OF WHEAT RFLP LOCI

HART G E; TULEEN N A; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6957 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 88 to 31 AUG 90

Objectives: PROJ. 8800380. To determine the chromosome-arm locations of RFLP loci in T. aestivum cv: Chinese Spring (CS), to identify loci that display RFLPs among hexaploid wheat varieties, to initiate genetic mapping of wheat RFLP loci, and to determine the chromosomal locations of homologous RFLP loci in two wheat relatives.

Approach: Genomic DNA of CS aneuploids and wheat varieties will be purified, digested, electrophoresed, blotted onto membranes, and hybridized to genomic DNA clones. Loci will be assigned to chromosomes and arms in CS and wheat relatives based on intergenomic variation in RFLs. RFLPs detected among varieties will be used to map wheat loci.

Progress: 88/01 to 88/12. The objectives of this research are to determine the chromosome-arm locations and genetic-map positions of RFLP loci in hexaploid wheat and to determine the chromosomal locations of

homologous RFLP loci in two diploid Triticeae species. The research on hexaploid wheat has been initiated using gDNA probes and Southern blots containing genomic DNA from aneuploid lines and varieties. Several RFLP loci have been localized in wheat chromosomes and a small number of mutants suitable for use in genetic mapping studies have been identified during the three months that the research has been underway.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

17.033* REGULATION OF GENE EXPRESSION DURING ENDOSPERM DEVELOPMENT

OKITA T W; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO0590 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 90

Objectives: Determine the pathway(s) through which protein bodies are formed during wheat endosperm development. Identify the molecular signals responsible for storage protein sorting and packaging within the cell. Determine the biochemical basis for the differential recruitment of rice glutelin and prolamine mRNA transcripts into translational complexes. Elucidate the gene structure of endosperm specific ADPglucose pyrophosphorylase.

Approach: Protein body formation during wheat endosperm development will be evaluated by electron microscopy and immunocytochemical techniques using gliadin and glutelin antibodies. For protein sorting studies, DNA constructs containing the coding segment of the gliadin and glutelin storage proteins fused to a reporter gene will be transferred into tobacco and the cellular site(s) of accumulation evaluated in different tissues. To identify peptides responsible for intracellular transport, successive 3'-end deletions of the storage protein coding segment will be performed, fused to a reporter gene and transferred into tobacco. Rough endoplasmic reticulum fractions enriched for glutelin and prolamine synthesis will be attempted using differential and sucrose density gradient centrifugation.

Progress: 88/01 to 88/12. Gel retardation studies have shown that nuclear proteins from developing wheat endosperm bind specifically to a gliadin promoter fragment, -113 bp to -231 bp. This fragment contains both a CCAAT box and CACA motif conserved among several cereal seed protein genes. By isolating the appropriate restriction DNA fragments, specific binding activity to both sequence motifs with wheat endosperm extracts has now been demonstrated. In transient expression assays, deletion of this DNA segment results in substantial reduction in gliadin promoter activity. Hence, the cumulative information from both the transient expression of the gliadin promoter and gel retardation studies indicates that the CACA and/or nearby DNA sequences are important

for transcription and the gliadin genes. Immunocytochemical-electron microscopy studies showed that, unlike the prolamines of maize, rice and sorghum, the prolamines of wheat are packaged via the Golgi complex. In many instances, small electron dense vesicles containing proteinaceous material reacted to gliadin antibodies can be observed directly connected to the distal ends of the Golgi cisternae. The structure of the rice prolamine polypeptide was elucidated by analysis of recombinant DNA clones. Unlike all other cereal prolamines analyzed to date, the rice prolamine gene and encoded protein are devoid of repeating DNA and peptide segments, respectively.

Publications: 88/01 to 88/12
KIM, W.T. and OKITA, T.W. 1988. Nucleotide and Primary Sequence of a Major Rice Prolamine. FEBS Lett. 231:308-310.
KIM, W.T., KRISHNAN, H.B., FRANCESCHI, V. and OKITA, T.W. 1988. Formation of Wheat Protein Bodies: Involvement of the Golgi Apparatus in Gliadin Transport. Planta

176:173-182.

KIM, W.T. and OKITA, T.W. 1988. Structure, Expression and Heterogeneity of the Rice Seed Prolamines. Plant Physiol 88:649-655.

CM 18 OTHER SMALL GRAINS

18.001 CRISO048391
GERMPLASM ENHANCEMENT AND IMPROVED BREEDING
METHOD S IN BARLEY

RAMAGE R T; Agricultural Research Service; Agricultural Research Service, Tucson, ARIZONA 85721.

Proj. No.: 5342-21220-001-00D

Project Type: INHOUSE Agency ID: ARS Period: 25 MAY 83 to 25 MAY 88

Objectives: Conduct research on the genetics and cytogenetics of barley in order to enhance germplasm and improve methods of breeding barley.

Approach: The genetic and cytogenetic program includes inheritance studies of qualitative and quantitative characters, allele testing, chromosome mapping, studies of duplications and deficiencies, translocations, inversions, aneuploids, polyploids, desynaptics, and studies of extra chromosomal inheritance. Breeding methods include mutation studies compositve crosses evolutionary breeding male sterile facilitated recurrent selection gamete selection and commercial hybrids from balanced tertiary trisomics and from cytoplasmic male sterility. Enhancement of genetic populations include developing short strawed, lodging resistant spring and winter barley populations populations with multiple disease resistance early barleys for use in double cropping systems barleys with a wide range of adaptation and barleys designed to perform under stress conditions such as being grown with limited and brackish irrigation water.

Progress: 87/01 to 87/12. The order of dominance of, and the effect of background genotype on, four short-awn alleles at the 1k2 locus was studied. Seed stocks of a set of primary trisomics in 'Betzes' was increased for distribution. The reduced-water-use population CC XXXIX is being backcrossed into cytoplasms of Hordeum spontaneum collections from dry areas of the Middle East. Composite crosses and male sterile facilitated recurrent selection populations are being continued and improved. CC XXXII is being developed for maximum yield under irrigated, high-yield conditions by increasing lodging resistance, multiple disease resistance and tolerance, seed size and total biomass production while retaining short plant stature. Seed incorporating the latest improvements was made available to breeders in 87. CC XXXIX is being developed for superior performance with limited water or water of low quality. A line selected from CC XXXIX was named 'Seco' and released for such uses as erosion control, soil stabilization and weed control, especially on abandoned farm land. Cytoplasmic male sterility, sterility maintainers and fertility restorers are being established in CC XXXII-type populations for use in developing hybrid barleys. To reduce cost of production, shrunken endosperm genes are being backcrossed into a CC XXXII-restorer pop. that has msml sterile cytoplasm. Short straw, large seed, high biomass and yield winter barley populations are being developed.

Publications: 87/01 to 87/12

Ramage, R.T. 1986. Alleles at the short-awned locus 1k2 on chromosome 1 and 1k5 on chromosome 4. Barley Genetics Newsletter 16:22-23.

Ramage, R.T. 1987. A history of barley breeding methods. Plant Breeding Reviews 5:95-138.

Ramage, R.T. and R.K. Thompson. 1987.
Announcement of availability of new seed of Composite Cross XXXII. Barley Newsletter 30:41-42.

18.002 CRISO14304 RFLP METHODOLOGY FOR RELATEDNESS AMONG SMALL GRAINCEREAL ACCESSIONS

SHANDS H L; MIRCETICH S M; QUALSET C D; Genetic Resources Cons Program; Hunt Hall-university of Cal, Davis, CALIFORNIA 95616

Proj. No.: 5306-21220-001-015

Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 30 SEP 88 to 30 AUG 92

Objectives: A) Develop and superimpose the RFLP linkage map of the barley genome on existing linkage maps, B) Identify and map hypervariable regions in barley genomes, C) Compare variation in single-copy RFLP loci with variation in allozymes, and D) Determine relatedness among accessions from a barley collection.

Approach: A) Carry out RFLP mapping in an F2 population of Betzes x C13108, isolating DNA from several hundred individual F2 plants, B) use sequential hybridization strategy, involving probing restriction enzyme digests with selected clones, C) use previously studied lines from CCXXI, Iranian landraces, and H. spontaneum, D) use allozymes and seed proteins to determine relatedness of over 100 barley accessions with BYDV resistance. Documents Specific Cooperative Agmt with University of California, Davis.

18.003 CRISO083153

ALLARD R W IN THE GENUS AVENA; Genetics; University of California, Davis, CALIFORNIA

Proj. No.: CA-D*-GEN-4067-H Project Type: HATCH Agency ID: CSRS Period: 01 DEC 80 to 30 SEP 86

Objectives: To determine genome organization and evolution in the genus Avena at the DMA level of resolution: To correlate morphological characters, disease resistance, and other genetic characteristics of wild and domesticated oats with major repetitive DNA families and allozyme profiles.

Approach: The approach will be multi-disciplinary. Data will be collected and integrated from cytology, morphology, protein electrophoresis, and DNA analysis of wild and cultivated oats.

Progress: 80/12 to 86/09. Research reported earlier in this project established that specific combinations of ribosomal DNA spaces-length variants are correlated precisely with multilocus allozyme genotypes, and with habitat, in California. For example, a "xeric" genotype characterized by specific alleles for eight allozyme loci, and found exclusively in arid habitats is also fixed for rDNA spacer-length variants (alleles) 13, 10, 8 and 7 whereas a "mesic" genotype characterized by a different set of specific alleles for the eight allozyme loci is fixed for rDNA spacer-length variants (alleles 15, 9, 8, and 7). Studies in the present report period have focused on A. barbata from the Mediterranean Basin and especially on Spanish populations known to be ancestral to the colonial California populations. Results establish that all of the allozymes and rDNA alleles from the ancestral Spanish populations are present in California but that none of the multilocus genotypes of California are present in Spain. Thus, during 200 years of evolution in California, selection has arranged the basic genetic materials introduced from Spain into novel sets of coadapted complexes, each precisely correlated with specific habitats in California. Three manuscripts reporting these results are under preparation for publication in Genetics and the Proceedings of the National Academy of Sciences.

Publications: 80/12 to 86/09
NO PUBLICATIONS REPORTED THIS PERIOD.

18.004 CRISOO14667
MECHANISMS OF PATHOGENESIS AND RESISTANCE IN
PLANT-PARASITE INTERACTIONS

KEEN N T; SIMS J J; ENDO R M; Plant Pathology; University of California, Riverside, CALIFORNIA 92521.

Proj. No.: CA-R*-PPA-0865-H Project Type: HATCH Agency ID: CSRS Period: 03 OCT 85 to 30 SEP 90

Objectives: Elucidate the molecular mechanisms underlying the expression of single gene disease resistance in plants.

Approach: Screen libraries of Pseudomonas syringae pv. glycinea races for avirulence genes. Characterize genes by sequencing and translation into proteins in E. coli. Protein products will be isolated and used in assays to determine if they are the recognitional elements that interact with soybean resistance gene products. Using I labelling of the pathogen protein, isolate plant resistance gene proteins. Use plant proteins as antigens for antiserum production, which could be used to isolate the plant disease resistance gene from a phage library of plant DNA.

Progress: 88/01 to 88/12. The complete structure of four pel genes encoding pectate lyases from Erwinia chrysanthemi was published. Work was also completed on sequencing a new pectate lyase gene from Erwinia carotovora and this work is now in press and pending publication. Significantly, the new gene and a related gene from Yersinia pseudotuberculosis

are considerably different from the pel genes of E. chrysanthemi. In other work, the structures of two additional avirulence (avr) genes was published during the year and the sequencing of an additional gene, avrD, has been completed. This latter gene has been shown to function in Escherichia coli cells to cause them to elicit the hypersensitive reaction in the proper soybean cultivars. Furthermore, culture fluids of the E. coli cells have been shown to contain a low molecular weight elicitor that also elicits the HR in these soybean cultivars. In collaborative work with Dr. Mark Stayton at the University of Wyoming, we are attempting to deduce the structure of this elicitor. It is believed that the information will give significant indications regarding the function of avrD in pseudomonads from which it has been cloned. The work also encourages us to attempt the cloning of the plant disease resistance gene complementing avrD.

Publications: 88/01 to 88/12

STASKAWICZ, B., DAHLBECK, D., KEEN, N.T. and NAPOLI, C. (1987). Molecular characterization of cloned avirulence genes from race O and race 1 of Pseudomonas syringae pv. glycinea. J. Bacteriol. 169:5789-5794.

SCHLEMMER, A.F., WARE, C.F. and KEEN, N.T. (1987). Purification and characterization of a pectin lyase produced by Pseudomonas

169:4495-4498.

MANULIS, S., KOBAYASHI, D.Y. and KEEN, N.T. (1988). Molecular cloning and sequencing of a pectate lyase gene from Yersinia pseudotuberculosis. J. Bacteriol. 170:1825-1830.

fluorescence W51. J. Bacteriol.

18.005* CRISO044250 EVOLUTION OF POLYPLOID WHEATS VIA AMINO ACID SEQUENCING AND ELECTROPHORETIC STUDIES

KASARDA D D; CALDWELL K A; FULLINGTON J G; Agricultural Research Service; Western Regional Res Center, Albany, **CALIFORNIA** 94710. Proj. No.: 5325-20520-011-00D

Project Type: INHOUSE Agency ID: ARS Period: 12 JAN 78 to 31 MAR 86

Objectives: Determine the species that contributed genomes to polyploid wheats, the changes in these genomes that resulted from polyploid formation or subsequently, and the consequences of thee factors for wheat quality..

Approach: Storage proteins from diploid species related to wheat, especially those of the genera Triticum and Aeuilops, and trom tetraploid and hexaploid wheats will be fractionated into less complex mixtures or individual components for elctropphoretic characterization and amino acid sequencing. High-performance liquid chromatography as well as traditional methods of gel filtrationand ion exchange chromatography will be used for protein and peptide purification. Both one-dimensional and two-dimensional methods of electrophoresis will be used. Sequencing will be carried out by

automatic and manual techniques. Results will be analyzed in relation to protein structure and gene evolution and the potential for quality improvement or maintenance in wheat through, or in conjunction with, introduction of alien genetic material into the wheat genome..

Progress: 86/01 to 86/03. The minor omega-gliadin component coded on chromosome 1A of bread wheats was purified and characterized by N-terminal amino acid sequencing to demonstrate close relationship of the gene coding for this protein and similar proteins from the species Triticum monococcum. This protein is of special interest in defining gene clustering in wheat because it has been reported to show significant recombination relative to other omega gliadins.

Publications: 86/01 to 86/03 NO PUBLICATIONS REPORTED THIS PERIOD.

18.006 CRISO009906 GENETIC AND LINKAGE STUDIES IN BARLEY

TSUCHIYA T; Agronomy; Colorado State
University, Fort Collins, COLORADO 80523.
Proj. No.: COLO0323 Project Type: HATCH
Agency ID: CSRS Period: O7 OCT 83 to 30 JUN 88

Objectives: Overall objective is chromosome engineering in genetics andbreeding in barley. To study the behavior of genes, their interrelationships and inheritance, for improvement of linkage maps in barley. Collection, maintenance and cataloging of genetic and trisomic stocks used in the genetic and linkage studies in barley. Development of 3-5 multiple genetic marker stocks, for each of 14 chromosome arms of seven barley chromosomes.

Approach: Cytogenetic methods will be used to study genetic architecture of barley chromosomes. Primary, secondary and telosomic trisomics, and trisomics with extra acrocentric and other fragment chromosomes will be used in crosses with various genetic marker stocks and new mutant lines. Various types of trisomic analyses, especially primary, telosomic and acrosomic trisomic analysis will be used to associate genes with chromosome, chromosome arm and chromosomal segments. Conventional genetic analysis is also used for final step in linkage mapping.

Progress: 88/01 to 88/12. Telotrisomic analysis was conducted with seven telotrisomic lines (Triplo 1L, 1S, 3S, 5L, 6S and 7S) and some 25 genes. Combining previous and new results, the centromeres were located in all seven genetic linkage maps of barley with at least one gene located in each of 14 chromosome arms. The effect of the short arm of barley chromosomes was studied with five arms. The effect was evaluated in four short arms (3S, 5S, 6S, 7S) in monotelosomic condition and five ditelotrasomics (Telo 2S, 3S, 5S, 6S, 7S). Triplo 2S ditelotetrasome showed exaggerated effect of a single dose of 2S in the character. However, ditelotetrasomes for other four telosomes (3S, 5S, 6S, 7S) were morphologically

almost normal with no or almost no qualitative effect, though some showed quantitative effect. Three new acrotrisomics, (Triplo 5S(superscript 5L), 6S(superscript 6L) and 7S(superscript 7L)) showed similar characteristics to corresponding primary trisomics indicating that intact proximal segment of the long arm of these three chromosomes carry morphogenetic or developmental genetic elements to control diagnostic characteristics of primary trisomics. A new barley line with 9 pairs of chromosomes (2n = 18) was established by combining two 8-paired barley lines, 12 + 2 (telo 1L + telo 1S) \times 12 + 2 (acro 3L(superscript 3S) + telo 3S) as follows: 2n = 18 = 10 + 2 (telos 1L + 1S + 3S + acro3L(superscript 3S) + telo 3S) = 9II.

Publications: 88/01 to 88/12
No publications reported this period.

18.007 CRISO033910 ANEUPLOID ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES

TSUCHIYA T; Agronomy; Colorado State
University, Fort Collins, COLORADO 80523.
Proj. No.: COLO8333 Project Type: CRGO
Agency ID: CRGO Period: O1 AUG 82 to 31 JUL 85

Objectives: Proj. No. 8200327. Genetic architecture of barley chromosomes will be studied by means of trisomic analyses.

Approach: Primary trisomic analysis will be used to associate mutant genes with respective chromosomes. Mutant genes associated with respective chromosomes are cytogenetically studied by using ten available telosomic trisomics (IL, IS, 2L, 2S, 3L, 3S, 4L, 5L, 6S, 7S). The telotrisomic analysis provides information on gene-chromosome arm relationships and the location of the centromere in genetic linkage maps. Linkage maps in each arm of seven chromosomes, and morphological and physiological characteristics are studied in detail. These comparative studies will provide some insight into the genetic architecture of chromosome and chromosome arms of barley.

Progress: 86/01 to 86/12. Telotrisomic analysis: Eleven available telotrisomic lines covering all seven barley chromosomes showed that all telocentric chromosomes for the long arm (1L, 2L, 3L, 4L, 5L) had same as or very similar effects on morphological characteristics to the corresponding primary trisomics. However, all telosomes for the short arm (1S, 3S, 5S, 6S, 7S), with the exception of 2S, showed almost no effect on the qualitative characters of telotrisomics resembling the normal disomic plants. These results suggest that morphogenetic or developmental genetic elements controlling the expression of various qualitative characters are located in the long arm of most of barley chromosomes, while the short arms devoid of such genetic elements. Acrotrisomic analysis: Detailed study of several acrotrisomics (Triplo 1L S, 3L S, 4L L S. 5S L. 6S L & 7S L) showed that the above-mentioned morphogenetic or developmental

genetic elements may be located in approximately 30% proximal segment of chromosomes. Genetic analysis of various genes with acrotrisomics provided some interesting information on the location & orientation of genes. One interesting case is the orientation of a1 and yst2 in the short arm of chromosome 3. The orientation of these two genes in the present map may have to be reversed; from Centromere - a1 - yst2 to Centromere - yst2 - a1. Usefulness of acrotrisomics has been shown in some cases including the example mentioned above. Project Terminated July 1985. The above work was completed during the project period but it was not reported.

Publications: 86/01 to 86/12
SHAHLA, A. and TSUCHIYA, T. 1986.
Cytogenetics of acrotrisomic 5S_5_L in barley. Can. J. Genet. Cytol. 28.
TSUCHIYA, T., SHAHLA, A., and HANG, A. 1986.
Acrotrisomic analysis in barley.
Proc. 5th Intern. Barley Genet. Symp.,
Okayama, Japan. (In press).
TSUCHIYA, T. 1986. Chromosome engineering in aneuploid barley. Proc. 1st Intern. Symp.
Chromosome Engineering in Plants, Xian,
China. (In press).
FURUTA, Y. and TSUCHIYA, T. 1986. A new compensating trisomic and tetrasomic for chromosone 4 in barley. Barley Genet.

18.008 CRISO137480 BARLEY GENETICS AND PLANT CYTOGENETICS

Newslett. 16.

TSUCHIYA T; HANG A; WANG S; Agronomy; Colorado State University, Fort Collins, ${\bf COLORADO}$ 80523.

Proj. No.: COLOO625 Project Type: HATCH Agency ID: CSRS Period: 14 FEB 89 to 30 JUN 93

Objectives: The overall objective of this project is basic genetic and cytogenetic studies in various plant species for aiding the progress in genetics and their direct and/or indirect uses in plant breeding programs.

Approach: We use chromosome manipulation approaches in most of the research work. For barley genetics chromosomal mutants, mainly various types of trisomics and many genetic mutants are used to improve genetic linkage maps and study the genetic architecture of barley chromosomes. For other materials, karyotype analysis by conventional and/or Giemsa-banding techniques of chromosome studies will be used.

18.009 CRISO135752 EXPRESSION OF ENDOGENOUS AND INTRODUCED PHYTOCHROME GENES IN BARLEY

COLBERT J T; WUNDER B A; Biology; Colorado State University, Fort Collins, ${\bf COLORADO}$ 80523.

Proj. No.: COLR-5-31403 Project Type: CRG0 Agency ID: CRGO Period: O1 JUL 88 to 30 JUN 90 Objectives: Isolate and characterize phytochrome cDNA clones from barley; 2) quantify the light-induced down-regulation of phytochrome mRNA abundance in etiolated barley seedlings; 3) develop a hybridization probe capable of specifically detecting oat phytochrome mRNA in the presence of barley phytochrome mRNA; and 4) produce a construct containing an oat phytochrome gene transcriptionally fused to a constitutive promoter.

Approach: Barley phytochrome cDNA clones will be isolated from a barley cDNA library. The library will be screened with radiolabelled oat phytochrome sequence. The longest barley phytochrome cDNA clones will be characterized by restriction mapping and DNA sequencing. Quantitation of barley phytochrome mRNA levels will be accomplished by using radiolabelled RNA, derived from the barley cDNA clones, to probe slot blots. Information obtained from sequencing the barley phytochrome cDNA clones will be used to develop a probe that hybridizes specifically to oat phytochrome mRNA. To produce the chimaeric oat phytochrome gene we plan to make use of an already available truncated phytochrome gene that lacks the promoter region. This phytochrome gene will be fused to a constitutive promoter (currently we plan to use the CAMV 35s promoter) and inserted into a vector containing a selectable marker.

18.010 CRISO098914 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT

SMITH R L; Agronomy; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-AGR-O2317 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 88

Objectives: Identification and Characterization of Agriculturally Important Genetic Systems, Regulation of Gene Expression and the Delivery of Genetic Material to Higher Plants and Associative Microorganisms, and Somatic Cell Genetics and Plant Development: The Modification, Selection, Regeneration, and Propagation of Plants through Cell and Tissue Culture.

Approach: Compare organelle DNA isolated from several sterile cytoplasms to fertile (B lines) and revertant counterparts using restriction endonuclease and southern blot hybridization patterns. Sequences that differ in those comparisons will be cloned and characterized in detail. Gene products of those clones will be characterized. Total plant DNA will be isolated from selected plant genotypes and southern blotted. Probes will be constructed from cDNA libraries and from random genomic clones. Those probes will be evaluated for their expression of RFLPs. Those RFLPs will be correlated with desired traits and tested for linkages.

Progress: 87/10 to 88/09. The chloroplast (ct) and mitochondrial (mt) DNAs were isolated from two rice subspecies, Oryza sativa,

japonica and indica and compared by restriction endonuclease analysis. Similarly, P.I. 353705 mtDNA was compared with the mtDNA of its long term suspension culture to determine genomic stability during culture. Chloroplast DNA of the two subspecies showed variation with only two of eleven endonucleases tested, whereas their mtDNAs showed considerable variation with five endonucleases. That data indicated that chloroplast genomes are more highly conserved than mitochondrial genomes. Relative values of restriction and hybridization patterns for study of phylogenetic relationships were evaluated. Only minor mtDNA variation produced by the long term suspension culturing. Molecular genetic studies on fatty acid regulation in peanut were initiated. Of the three DNA isolation methods evaluated, the CTAB precipitation method appeared work best giving high yields of good quality and each of those clones recognized peanut DNA sequences. Lambda libraries are being prepared to isolate clones of the sequences with nucleotide similarities to the rat liver desaturases.

Publications: 87/10 to 88/09
CHOWDHURY, M.K.U., SCHAEFFER, G.W., SMITH,
 R.L. and MATTHEWS, B.F. 1988. Molecular
 analysis of organelle DNA of different
 subspecies in rice and the genomic
 stability of mtDNA in tissue cultured cells
 of rice. Theor. Appl. Genet.

18.011* CRISO143897 EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO

HOFFMAN D L; BOCKELMAN H E; Agricultural Research Service, Aberdeen, **IDAHO** 83210. Proj. No.: 5366-21000-004-00D

Project Type: INHOUSE Agency ID: ARS Period: 20 DEC 88 to 19 DEC 93

Objectives: To determine the feasibility of DNA fragment length polymorphism for genetic analysis & germplasm enhancement in oats. To initiate a linkage map of oats using conventional and molecular markers. To begin determining linkages or associations of molecular markers with economic genes in oats. Evaluate wheat germplasm for vesicular arbuscular mycorrhizal dependency. Develop bioassays to detect allelochemicals in wheat germplasm. Coordinate Uniform Eastern and Southern Soft Red Winter Wheat Nurseries.

Approach: A genomic DNA library of oats has been constructed and clones from this library will be evaluated for copy number. Low copy clones will be screened for polymorphism among genetically diverse oat cultivars and related wild species. Linkage or association of polymorphic markers will be assigned to chromosome with a monosomic series. Linkage among markers will be determined using recombinant inbred populations generated from a wide cross in oats. Wheat germplasm will be evaluated for vesicular/arbuscular mycorrhizae dependency in two steps: 1)selection of accessions with rapid and substantial colonization as a preliminary screen and then

2) evaluation for growth response to colonization under low phosphorus conditions. Bioassay techniques to detect allelochmicals in wheat germplasm will be developed by studying the effects of exudates on germination, seedling growth, dry matter production, etc. of various indicator species. IBC Approval Pending.

18.012 CRISO011779 PLASMIDS IN PLANT PATHOGENIC BACTERIA

SHAW P D; Plant Pathology; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.

Proj. No.: ILLU-68-0373 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To characterize genetic determinants for phage reception, transfer, and mobilization on plasmid pBPW1 from Pseudomonas syringae pv. tabaci BR2; to characterize the genetic determinant of an outer membrane protein located on that same plasmid; to assess the role of pBPW1 in plant disease; and to characterize pathogenicity genes on the BR2 chromosome.

Approach: Plasmid genetic determinants will be localized by transposon mutagenesis, and plasmid restriction fragments encoding the determinants will be cloned. The cloned fragments will be used to determine gene functions. Pathogenicity genes on the chromosome will be located by transposon mutagenesis, and the cloned mutant genes will be used as probes to isolate functional genes from the BR2 parent. These clones will be characterized structurally and genetically.

Progress: 87/01 to 88/09. Studies have begun on the regulation of expression of pathogenicity genes in plant-pathogenic bacteria. When Tn5 mutants of Pseudomonas syringae pv. tabaci (which were nonpathogenic on tobacco) were inoculated onto leaves of Phaseolus vulgaris (cv. Top Crop), disease symptoms were observed. In some experiments, bacteria reisolated from beans had regained pathogenicity on tobacco, but in other experiments they remained nonpathogenic. In all cases, however, probing restrictions digests of DNA from the reisolated bacteria with labeled In5 coupled with growth experiments, indicated that genetic rearrangements had occurred during growth in the bean plants. In one experiment, the transposon (and probably flanking sequences) had been deleted. Our results indicated that the rearrangements were induced by some plant factors. In preliminary experiments to test this, Tn3-HoHol mutants of a 7.2 kb fragment that restores pathogenicity to one of the mutants have been constructed, and expression of a promotorless lacZ gene in the transposon is being tested. Similarly, Tn3-HoHo1 mutants of a 10 kb HindIII fragment that restores pathogenicity to a nonpathogenic mutant of Xanthomonas campestris pv. glycines have also been constructed. Cells containing plasmids with insertions of the transposon in both possible orientations at sites known to

contain the pathogenicity genes, fail to produce (beta)-galactosidase in culture. These results indicate that expression of the pathogenicity gene(s) might be inducible by plant factors.

Publications: 87/01 to 88/09
No publications reported this period.

18.013 CRISO089981 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT

SHAW P D: Plant Pathology; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.

Proj. No.: ILLU-68-0325 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 82 to 30 SEP 88

Objectives: Identification and Characterization of Agriculturally Important Genetic Systems.

Approach: Restriction endonuclease maps will be prepared of the plasmids. These maps will be used as bases for constructing genetic maps, making use of restriction fragments cloned into appropriate vehicles, and transposon mutagenisis. Clones will be characterized by complementation studies, and genes involved in pathogenicity will be characterized.

Xanthomonas Progress: 87/10 to 88/09. campestris pv. glycines (XCG) causes bacterial pustule on soybeans. A soybean cotyledon assay was developed to assay pathogenicity. Fifteen nonpathogenic mutants of strain 8ra were isolated after treatment of the parent with NTG and examining about 2000 individual colonies. Auxotrophic and pigmentless mutants were also obtained. A genomic library was constructed by partial Sau3A1 digestion of total DNA from 8ra and ligating the fragments into pLAFR3. The library was transferred to XCG mutant NP1, and one transconjugant was able to restore pathogenicity to the mutant. It was also able to complement three of the other 15 mutants. A restriction map was prepared of the cloned 30 kb fragment, and deletion analysis indicated that genes that were capable of restoring pathogenicity to NP1 resided on a ten kb HindIII fragment. This fragment was subcloned and subjected to Tn3-HoHol mutagenesis. The insertion mutants were transferred into NP1, and a region of about 2.5 kb within a 2.7 kb Clal fragment was defined as the region containing the pathogenicity genes(s). A 2.2 kb Clal-BglII fragment was subcloned into pW5A, and conjugated into NP1. It did not restore pathogenicity; thus, a region of about 200-300 bp between the BglII restriction site and a Tn3-HoHol insertion appears to be important for gene function. The 2.7 kb Clal fragment has been cloned into pW5A and transferred into NP1. Transconjugants are being tested for pathogenicity, and the fragment is being sequenced.

Publications: 87/10 to 88/09
HUANG, I., LIM, S. M., and SHAW, P. D.
 (1987). Soybean cotyledon bioassay for
 detecting nonpathogenic mutants of
 Xanthomonas campestris pv. glycines.
 Phytopathology 77:1709.

HUANG, I., LIM, S. M., and SHAW, P. D. (1988). Generation and complementation of pathogenicity genes of Xanthomonas campestris pv. glycines 8ra. J. Cellular Biochem. 12C:252.

18.014 CRISO131014 STRUCTURE AND EXPRESSION OF PLANT GENES ENCODING CALMODULIN

ZIELINSKI R E; Plant Biology; 809 South Wright Street, Champaign, **ILLINOIS** 61820.

Proj. No.: ILLR-86-1932 Project Type: CRGO Agency ID: CRGO Period: O1 JUN 86 to 31 MAY 88

Objectives: PROJECT 8600051. The objectives of this research are to understand the organization and expression of genes encoding calmodulin in barley. We will also explore the possibility that Ca-binding proteins related to calmodulin exist in plant cells.

Approach: Our approach to this research problem consists of constructing cDNAs from barley leaf mRNA and screening these recombinant molecules using a cDNA made against eel calmodulin mRNA as a hybridization probe. Homologous plant calmodulin cDNAs thus obtained will be characterized by DNA sequencing, and used as molecular probes to isolate cloned structural gene sequences. These cloned structural gene sequences. These cloned structural genes will be characterized by both physical mapping with restriction enzymes and DNA sequencing. Calmodulin gene expression will be examined using homologous cDNA probes to screen gel-fractionated nuclear and cytoplasmic RNA fractions.

Progress: 87/01 to 87/12. The aims of this project are to clone DNA sequences encoding plant calmodulin (CaM) and to examine the regulation of CaM gene expression during barley leaf development. Using a heterologous CaM cDNA probe, we established that CaM is encoded by an mRNA of about 650+/-100 nucleotides in barley and pea. In the naturally occurring developmental gradient from the base to the tip of barley leaves, steady-state CaM mRNA levels show little variation except in the basil, meristematic mRNA population, where they are three-to- four-fold higher than elsewhere in the leaf. Etiolated barley leaves appear to contain about two-fold higher relative steady-state CaM mRNA levels than fully green leaves, indicating that CaM mRNA accumulation is down-regulated by light. Poly A RNA from etiolated barley was found to contain additional mRNAs that may encode CaM-like proteins. The steady-state levels of these CaM-like mRNAs decrease to below the limit of detection during light-induced development. Current studies support the idea that etiolated barley leaves and meristematic zones of green leaves contain somewhat higher steady-state levels of CaM mRNA and protein than do non-dividing fully green leaf cells. In addition, these experiments revealed the presence of 7 potential CaM-like Ca -binding proteins. The steady-state level of each of these p lypeptides is negatively regulated by light. To confirm our studies, we have cloned cDNAs encoding barley leaf CaM mRNA.

Publications: 87/01 to 87/12

ZIELINSKI, R.E. 1987. Calmodulin mRNA in Barley (Hordeum vulgare L.): apparent regulation by cell proliferation and light. Plant Physiol. 84: 937-943.

Plant Physiol. 84: 937-943.

LADROR, U.S. 1986. ATPase and protein kinase in membranes from corn roots: regulation of tonoplast ATPase by calmodulin or by protein kinase Ph.D. Thesis Univ. Illinois, Urbana, IL. 130 p.

18.015 CRISO033940 ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS

LARKINS B; WALBURG G; Botany & Plant Pathology; Purdue Res Foundation, West

Lafayette, INDIANA 47907.

Proj. No.: INDO55044B Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 82 to 31 AUG 84

Objectives: Proj. No. 8200759. Determine the primary structure of oat seed globulin proteins. Analyze the structural relationships between these proteins and the storage globulins in legumes, and determine if similar proteins exist in other cereals. Characterize the mechanisms regulating the synthesis and deposition of these proteins in the developing oat seed.

Approach: Messenger RNAs encoding oat globulins will be isolated from developing seeds. This mRNA will be used to construct recombinant cDNA clones. After isolating full-length cDNA clones, their DNA sequence will be analyzed to determine the primary amino acid sequences of the protein. These clones will also be used to study the transcription of the mRNAs and their level and subcellar distribution during seed development.

Progress: 85/01 to 85/12. Oat seeds are unusual among cereals because they contain very little (5-8%) prolamine protein. Instead, they primarily contain a saline-soluble globulin that has many of the structural characteristics of the storage globulin found in legume seeds. To better characterize the globulin protein we have constructed and characterized cDNA clones corresponding to globulin mRNAs. We have determined the DNA sequence of one clone that encodes the entire basic polypeptide and part of the acidic polypeptide of a globulin subunit. This analysis showed a great deal of homology between the basic polypeptide of the oat globulin and the basic polypeptide of the soybean globulin. Cross-hybridization analysis between different oat globulin cDNA clones indicate that the genes encoding these proteins are highly homologous. From Southern hybridization analyses we determined that the genes encoding these proteins are highly reiterated in the genome. This contrasts with the situation in legumes, where globulin genes are present in only 2-8 copies.

Publications: 85/01 to 85/12

NO PUBLICATIONS REPORTED THIS PERIOD.

18.016 CRISO097070 ANALYSIS OF SEED GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS

LARKINS B A; Botany & Plant Pathology; Purdue University, West Lafayette, INDIANA 47907.

Proj. No.: INDO55044-G Project Type: CRGO Agency ID: CRGO Period: 01 SEP 85 to 29 FEB 88

Objectives: Proj 8502826. The objectives of the proposed research are to construct and characterize recombinant DNA clones corresponding to the globulin and avenin mRNAs of oat seed. These clones will be used as probes to characterize the structure and expression of the genes encoding oat globulin and avenin proteins. Our overall objective is to determine the molecular basis for the differential expression of these genes during seed development. We will correlate gene numbers with levels of mRNA to determine if the expression of these genes is regulated primarily at the level of transcription.

Approach: We will eventually attempt to identify DNA sequences responsible for the developmental regulation of these genes. Recombinant cDNA clones will be synthesized with mRNA from developing oat seed. Synthetic oligonucleotide probes will be constructed based upon amino acid sequences of the seed proteins. The oligonucleotides will be radioactively labeled and used as probes to identify the corresponding sequences among the cDNA clones. These cDNA clones will then be used to isolate the corresponding genes from a genomic library.

Progress: 87/10 to 88/09. Oats are unusual among cereals in that the endosperms of their seeds contain only small amounts of prolamine proteins. Oat seeds instead contain large amounts of an 11S globulin similar to the one found in the embryos of dicot seeds. The oat globulin appears to share structural features of the dicot globulin, but it is much less soluble. To better characterize the structure of this protein and the genes encoding it, as well as investigate the molecular mechanisms responsible for the high proportion of globulin relative to prolamine synthesis, we have constructed and isolated cDNA and genomic clones of these oat storage protein genes. Sequence analysis of a globulin gene reveals that it is structurally very similar to its counterpart in dicots. These genes, which are present in 7-10 copies per genome, contain three introns and four exons at similar locations to genes found in dicots. The proteins have neutral amino acids rather than acidic ones at the COOH-terminus of the acidic polypeptide encoded by this gene and this appears to be responsible for the reduced solubility of the oat globulin. We have recently obtained clones of oat prolamine proteins, avenins. As is true of other cereal prolamines, these proteins contain no lysine. Experiments are in progress to characterize the relative number and transcription of these aenes.

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Publications: 87/10 to 88/09

SHOTWELL, M.A., AFONSO, C.A., DAVIES, E., CHESNUT, R.S., and LARKINS, B.A. Molecular characterization of oat seed globulins. Plant Physiol. (1988) 87, 698-704.

18.017 CRISO089946 ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY

MUTHUKRISHNAN S; Biochemistry; Kansas State University, Manhattan, KANSAS 66506.

Proj. No.: KANOO450 Project Type: STATE Agency ID: SAES Period: O1 JUL 83 to 30 JUN 86

Objectives: To study the organization of alpha amylase genes in barley genome with a view tounderstanding their regulation by plant hormones.

Approach: Genomic libraries of barley DNA fragments in bacteriophage Lambda will be screened to identify the clones containing alpha amylase sequences. These genomic clones will be mapped by digestion with restriction enzymes to localize the regions containing the coding sequences for Alpha amylases. The chromosomal location of barley Alpha amylase genes will be determined by Southern blot analysis of DNA prepared from addition lines containing a pair of barley chromosomes in a Chinese Spring wheat background. In situ hybridization of 32 P-labeled Alpha amylase clone cDNA will be done using cytological preparations from diploid, primary trisomic and monotelotrisomics of barley to confirm the results of Southern blot analysis and to identify the chromosome arm on which the Alpha amylase genes are located.

Progress: 83/07 to 86/06. Southern blot analyses of barley embryo DNA using an alpha-amylase cDNA clone probe revealed the presence of a family of 8-9 alpha-amylase genes in the barley genome. When a cDNA clone for the high pI group of alpha-amylases was used to probe DNA from wheat-barley chromosome addition lines, all of the six genes detectable by this probe were located on Betzes barley chromosome 6. Using a cDNA clone specific for the low pI group of alpha-amylases, all of the three bands detectable by this probe were found on barley chromosome 1. Alpha-Amylase genomic clones were isolated from a barley genomic library and one member of each group was sequenced. High pI alpha-amylase genes contained two introns while the low pI alpha-amylase genes contained three introns. The coding regions of low pI genomic and cDNA clones were closely related and differed from the coding regions of the high pI alpha-amylase genes by about 30%. The non-coding regions and 5'-upstream regions appeared to be unrelated. Multiple copies of repeat elements were found in the 5'-upstream regions of both the low pI and high pI alpha-amylase genes.

Publications: 83/07 to 86/06
MUTHUKRISHNAN, S., GILL, B.S., SWEGLE, M. and CHANDRA, G.R. 1984. Structural genes for alpha-amylases are located on barley chromosomes 1 and 6. J. Biol. Chem. 259:13637-13640.

KNOX, C. and MUTHUKRISHNAN, S. 1985. Barley genomic alpha-amylase genes characterized. Abstract PO-1-O19, First Int. Cong. Plant Molec. Biol., Savannah, GA. pp. 75.

18.018 CRISO098565 ORGANIZATION AND CONTROL OF THE HYDROLASE GENES IN BARLEY

MUTHUKRISHNAN S; Biochemistry; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KANO0624 Project Type: STATE
Agency ID: SAES Period: 01 JUL 86 to 30 JUN 89

Objectives: To identify DNA elements involved in regulation of hydrolase genes in barley.

Approach: Genomic clones for hydrolase genes that are known to be induced by gibberellic acid will be isolated by screening a barley genomic library using cDNA clones for GA-inducible sequences as hybridization probes. Clones showing positive hybridization will be purified and their DNA will be mapped with restriction enzymes. Regions coding for the hydrolase genes and those that contain the 5' regions of these genes will be identified. Fragments containing the 5' upstream sequences will be sequenced by the Sanger dideoxy procedure. Computer programs will be used to identify sequence elements that are common to all GA-inducible genes. These regions are likely to be involved in the regulation of hydrolase genes.

Progress: 88/01 to 88/12. A barley aleurone cDNA library was screened using P-labeled cDNA prepared by reverse transcription of mRNA from aleurone layers treated in the presence or absence of gibberellic acid (GA). A number of clones were identified whose transcripts were found in greater abundance in GA-treated tissue. Besides alpha-amylase cDNA clones, another set of clones representing an abundant mRNA in aleurone cells was identified. Messenger RNA hybrid-selected by a prototype clone of this group (clone 10) was translated in vitro to yield a 36 kilodalton protein. Analysis of the DNA sequence and the predicted amino acid sequence of the protein product of this clone indicates that this gene codes for a protein with homology to endochitinases from tobacco and bean. In addition, the predicted amino acid sequence includes a stretch that is closely related to a cyanogen bromide cleavage fragment from an endochitinase isolated from barley endosperm. The structural genes for endochitinase are present as multiple copies on barley chromosome 1. mRNA detectable by this clone increases in abundance in barley aleurone cells incubated in the absence and in the presence of gibberellic acid (GA), though somewhat elevated levels of this RNA are found in GA-treated cells. Western blot analysis of proteins from aleurone and endosperm tissues indicates the presence of multiple endochitinases differing in molecular sizes.

Publications: 88/01 to 88/12
RAHMATULLAH, R.J., HUANG, J-K., CLARK, K.L.,
CHANDRA, G.R. and MUTHUKRISHNAN, S.
Nucleotide and predicted amino acid

sequences of two different genes for high pI alpha-amylases from barley. Plant Molec Biol (In Press).

18.019 CRISCO98755 GENETIC MANIPULATION OF LACTIC DEHYDROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE

HANSON A D; Plant Research Lab; Michigan State University, East Lansing, **MICHIGAN** 48824. Proj. No.: MICLO1501 Project Type: HATCH Agency ID: CSRS Period: O1 JUL 86 to 30 JUN 91

Objectives: To isolate and characterize the anaerobically-induced lactate dehydrogenase (LDH) enzyme from barley, and using antibodies against this enzyme, and amino acid sequence data, to proceed to molecular cloning of the LDH genes of barley and other cereals; attempt to use the cloned LDH genes for manipulation of the flooding-sensitivity of roots.

Approach: The anaerobically-induced LDH activity of barley roots will be purified to homogeneity using affinity chromatography; antibodies will be raised in rabbits, and amino-acid sequencing of CNBr fragments will be carried out. The antibodies and oligonucleotides corresponding to the amino acid sequence data will be used to screen a cDNA library prepared from RNA isolated from anaerobic roots. Putative cDNA clones for LDH will be restriction mapped and partially sequenced to permit their identification. Initially the use of cloned LDH genes to modify flooding tolerance, the feasibility of anti-sense RNA approaches will be explored. if necessary in a model dicotyledonous species should routine transformation systems for monocotyledenous plants not become available soon. (If a model dicotyledonous plant is used, the barley LDH cDNA clones from barley will be used to isolate LDH clones from a cDNA library of the dicot species selected, and those dicot cDNA clones will be used to construct anti-sense genes for transformation experiments).

Progress: 88/01 to 88/12. This project concerns the function, genetic basis and regulation of anaerobically-induced lactate dehydrogenase (LDH) isozymes in barley and other cereals. The first step in our strategy towards understanding the anaerobic induction mechanism and the physiological function fo LDH is to obtain LDH cDNA closes. Oligonucleotide probes (17-mers) were synthesized based on the amino acid sequences of tryptic pepides of purified barley LDH. To minimize the degree of degeneracy, the oligonucleotides were synthesized as sub-pools and one sub-pool was selected based on its ability to hybridize to a poly (A) RNA species (on Northern blots) that was induced by hypoxia and was of sufficient size to encode LDH (1 bold center dot 7 kb). A cDNA library constructed in the plasmid pARC7 was screened with this probe; five positive clones were obtained. Two of these clones hybrid-selected poly (A) RNA which was (a) hypoxically induced and (b) had a cell-free translation product which was identical in size to LDH (40 kD) and was recognized by anti-LDH

serum. Sequencing of these two clones is in progress.

Publications: 88/01 to 88/12
HOFFMAN, N.E., HONDRED, D., HANSON, A.D. and BROWN, A.H.D. 1988. Lactate dehydrogenase isozymes in barley: polymorphism and genetic basis. J. Hered. 79:110-114.

18.020 OOO7368 BIOCHEMICAL AND DEVELOPMENTAL GENETICS OF HIGHER PLANTS

GENGENBACH B G; Agronomy & Plant Genetics; University of Minnesota, St Paul, **MINNESOTA** 55108

Proj. No.: MIN-13-032 Project Type: STATE Agency ID: SAES Period: 01 JUL 89 to 30 JUN 94

Objectives: Determine gene-enzyme relationships and isolate genes regulating amino acid biosynthesis, amyloplast development, and herbicide tolerance. Characterize mitochondrial transmission genetics and mutants. Develop tissue cultures of malting barley.

Approach: Specific genes will be isolated by molecular biology methods from normal and mutant genotypes to determine their role in amino acid biosynthesis, amyloplast development and herbicide tolerance. Progeny from plants containing heterogeneous mitochondrial genomes will be analyzed for molecular and phenotypic traits. Plants will be regenerated from barley tissue cultures and evaluated for malting quality, herbicide tolerance and agronomic traits.

Progress: 88/01 to 88/12. DNA and plant markers were used to prove that cytoplasmically-inherited mitochondrial (mtDNA) in corn can segregate through the female parent. Molecular analyses of individual maternal plants identified the relative proportions of two mtDNA arrangements known to be associated with T cytoplasm male sterility/disease susceptibility or male fertility/resistance. These analyses predicted the frequencies of corresponding contrasting plant phenotypes in the progeny. Rearrangements in a specific repeated mtDNA sequence adjacent to the atp9 gene were identified in four fertile mutants obtained from sterile S cytoplasm corn. S cytoplasm mtDNA was shown to have two atpA genes located in different regions of the genome. The genome organization of one atpA gene changed and the new version was amplified during a few generations of seed propagation indicating that mtDNA variation is established quickly. The cytochrome oxidase III gene from soybean mtDNA was isolated and sequenced. MtDNA polymorphisms among soybean lines derived from the Lincoln source of Mandarin germplasm were identified. Barley genotypes screened in the field and greenhouse for reaction to the herbicide imazethapyr exhibited differences, but no genotype was resistant. Selection for resistance in tissue cultures of Morex and comparisons of acetolactate synthase (herbicide target site) in sensitive and more tolerant genotypes have been initiated.

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Publications: 88/01 to 88/12

RINES, H.W., GENGENBACH, B.G., BDYLAN, K.L. and STDREY, K.K. 1988. Mitochondrial DNA diversity in oat cultivars and species. Crop Sci. 28:171-176.

PRING, D.R., GENGENBACH, B.G. and WISE, R.P. 1988. Recombination is associated with polymorphism of the mitochondrial genomes of maize and sorghum. Phil. Trans. R. Soc. Lond, B 319:187-198.

WANG, Y. 1988. Molecular analysis of mutants from maize cms-S fertile revertants. Ph.D. Thesis. Univ. Minnesota, St. Paul. 111p.

BURTON, J.D., STDLTENBERG, D.E., GRONWALD,

J.W., SDMERS, D.A., GENGENBACH, B.G. and WYSE, D.L. 1988. Inhibition of acetyl-coenzyme A carboxylase by sethoxydim and haloxyfop. Plant Physiol. 86s:111.

WANG, Y. and GENGENBACH, B.G. 1988. Unique mtDNA restriction fragment in an S cytoplasm fertile revertant line retaining the S1 and S2 plasmids. Miaze Genet. Coop. Newslett. 62:102-103.

GENGENBACH, B.G. 1988. Maternal segregation for a mtDNA deletion in maize. Genome 30 (supp.):315.

WANG, Y. and GENGENBACH, B.G. 1988. Fertile revertants of Scms maize have unique mtDNA fragments. Genome 30(supp.):315.

18.021* CYTOGENETICS IN PLANT IMPROVEMENT

CRISO006177

PHILLIPS R L; Agronomy & Plant Genetics; University of Minnesota, St Paul, MINNESOTA 55108.

Proj. No.: MIN-13-022 Project Type: HATCH Agency ID: CSRS Period: 01 DCT 87 to 30 SEP 92

Objectives: Develop somatic cell genetics systems for crop species; Design molecular genetic methods for transferring important traits; Elucidate and apply molecular and developmental cytogenetic information; develop genetic selection procedures for protein quality improvement.

Approach: Molecular and classical cytogenetic methods will be employed to test the basis of tissue culture-induced genetic variation; use molecular genetic markers to determine the chromosomal distribution of genes controlling agronomic traits; understand molecular biological basis of kernel growth; & design selection procedures for obtaining strains with improved protein quality.

Progress: 88/01 to 88/12. The gene regulating expression of a methionine-rich corn storage protein led to increased lysine levels when introduced into certain genetic backgrounds. A lysine + threonine laboratory seedling screening procedure was shown to detect altered ratios of methionine to lysine in corn kernels. DNA amplification in whole endosperms was documented by flow cytometry to achieve 3X the base DNA level. Differential replication of parts of the genome was shown not to occur. Ribosomal DNA (rDNA) in endosperm nucleoli was found to be less methylated than contiguous, non-nucleolar rDNA and represents specific rDNA sequences. Transposable element

(Ac) activation via the tissue culture process might be due to the activation of one of the 7 to 8 cryptic sequences shown to be present; one DNA segment co-segregates with Ac activity and a DNA methylation change is present in one case. Activation of a Spm element via tissue culture occurred in one instance. Molecular genetic probes were identified that distinguishes Tripsacum chromosomes in crosses with highly heterogeneous maize populations. A B73 genetic male-sterile system using duplicate-deficient chromosomes led to the production of an all male-sterile line (90 plants). Dat rDNA was localized to only the three satellited chromosomes. Chromosome 2 was shown to carry a distinct rDNA sequence. Little polymorphism for rDNA was shown for oat while related wild oat species are highly polymorphic.

Publications: 88/01 to 88/12

ARMSTRONG, C.L. and PHILLIPS, R.L. 1988. Genetic and cytogenetic variation in plants regenerated from organogenic and friable, embryogentic tissue cultures of maize. Crop Sci. 28:363-369.

LEE, M., GEADELMANN, J.L. and PHILLIPS, R.L. 1988. Agronomic evaluation in inbred lines derived from tissue cultures of maize. Theor. Appl. Genet. 75:841-849.

BENZIDN, G. and PHILLIPS, R.L. 1988. Cytogenetic stability of maize tissue cultures: A cell line pedigree analysis. Genome 30:318-325.

PHILLIPS, R.L., MCMULLEN, M.D., ENDMDTD, S. and RUBENSTEIN, I. 1988. Ribosomal DNA in maize. p. 201-214. In J.P. Gustafson and R. Appels (ed.) Chromosome Structure and Function: Impact of New Concepts.

KDWLES, R.V. and PHILLIPS, R.L. 1988. Endosperm development in maize. Intl. Rev. Cytol. 112:97-136. LEE, M.D. and PHILLIPS, R.L. 1988.

Chromosomal basis of somaclonal variation. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39:413-437.

PHILLIPS, R.L. and PESCHKE, V.M. 1988. Discovery of Ac activity among progeny of tissue culture derived maize plants. Intl. Symp. Plant Transposable Elements, Madison, WI p. 305-315.

18.022* TISSUE CULTURE GENETIC SYSTEMS

CRISO096427

PHILLIPS R L; RINES H W; Dffice of Research Admin.; University of Minnesota, St Paul, MINNESOTA 55108.

Proj. No.: MIN-8500192 Project Type: CRGD Agency ID: CRGD Period: O1 AUG 85 to 31 JUL 89

Objectives: Proj 8500192. Further document the occurrence of chromosomal and genetic variability in cell cultures and regenerated plants of oats and maize, test unifying hypothesis accounting for both chromosome breakage and new mutants, develop means of modifying cell genetic behavior, and evaluate possible applications of culture instability.

Approach: Experiments will test: a) the role of late replicating DNA and chromosome breakage in generating variability, b) the release of transposable elements during the tissue culture process, c) organogenic versus embryogenic cultures relative to stability and origin of plantlets, and d) the occurrence of gene transfer by chromosome exchange induced by the tissue culture process.

Progress: 88/01 to 88/12. Transposable genetic element (Ac) activated by the maize tissue culture process were studied at the molecular level. Cryptic Ac sequences were shown to be present in parental lines. A 10 kb Bal II DNA fragment homologous to Ac cosegregated with the new Ac activity. Altered DNA methylation was detected in one ${\sf Ac\text{-}positive}$ line. Approximately 700 tests for Spm activation via tissue culture revealed two cases of newly induced Spm activity. Agronomic evaluation of 269 oat lines derived from tissue culture-regenerated plants revealed both positive and negative variations for eight traits: height, heading date, grain yield, bundle weight, seed number, 100 seed weight, flag leaf area, and percent protein. Five lines showed significantly greater yield than the controls and 22 lines yielded significantly less Isoelectric focusing revealed banding pattern variation in avenin proteins among some of the lines. Fifteen haploid oat plants were recovered using embyro rescue following application of maize pollen to about 3000 emasculated oat florets. Cytological analysis of early stage embryonic and endosperm cells at division revealed lagging chromosomes, chromosomes not associated with mitotic figures, and numerous micronuclei. These observations support the suggestion that oat/maize hybrid zygotes are produced followed by elimination of the maize chromosomes during early embryo development.

Publications: 88/01 to 88/12

ARMSTRONG, C.L. and PHILLIPS, R.L. 1988. Genetic and cytogenetic variation in plants regenerated from organgenic and friable, embryogenic tissue cultures of maize. Crop Sci. 28:363-369.

LEE, M., GEADELMANN, J.L. and PHILLIPS, R.L. 1988. Agronomic evaluation of inbred lines derived from tissue cultures of maize. Theor. Appl. Genet. 75:841-849.

BENZION, G. and PHILLIPS, R.L. 1988. Cytogenetic stability of maize tissue cultures: A cell line pedigree analysis. Genome 30:318-325.

PHILLIPS, R.L., SOMERS, D.A. and HIBBERD, K.A. 1988. Cell/tissue culture and in vitro manipulation. In Corn and Corn Improvement (ed G.F. Sprague). Am. Soc.

Agron., Madison, WI (In press).
LEE, M.D. and PHILLIPS, R.L. 1988.
Chromosomal basis of somaclonal variation.
Ann. Rev. Plant Physiol. Plant Mol. Biol.
38:413-437.

PHILLIPS, R.L. and PESCHKE, V.M. 1988.
Discovery of Ac activity among progeny of tissue culture-derived maize plants. Intl. Symp. Plant Transposable Elements, Madison, WI p. 305-315.

SOMERS, D.A., PHILLIPS, R.L. and RINES, H.W. 1988. Corn and oat tissue cultures and genetic variation in regenerated plants. FFTC Symp. on Cell and Tissue Culture in Field Crop Improvement. Tsukuba, Japan (In press).

18.023* CRISO049430 GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS

STEINER W M M; Parasite-predator Bio & Ecol Bio Control of Insects Lab; Agricultural Research Service, Columbia, **MISSOURI** 65211. Proj. No.: 3622-24000-006-00D

Project Type: INHOUSE Agency ID: ARS Period: O1 OCT 84 to 30 SEP 89

Objectives: Genetically map and characterize chromosomal, morphological, DNA, and allozyme variants of selected entomophagous insects and determine their physiological, developmental and behavioral significance and their potential for utilization in bioengineering programs to enhance entomophageefficiency.

Approach: Populations of selected entomophages will be assayed using classical gene- tic techniques and restriction enzyme (DNA) technology to establish gene- tically typed isolines. These will be used to establish linkage maps and study genome structure in the context of predator-parasite/prey models. About 80 % of effort will be devoted to the model offered by Heliothis zea and its entomophages with 20% of effort reserved to develop genetic know- ledge of other models. Selected variants will also be biochemically char- acterized and their distributions in natural populations determined. Lab- oratory tests of physiological, developmental and behavior response under stresses posed by pesticides, temperature and desiccation will reveal whichphenotypes can enhance natural population fitness characteristics. Methods of transferring desirable traits will be developed.

Progress: 88/01 to 88/12. Selection for insecticide resistance in females of the parasitoid Microplitis croceipes was initiated to determine how amenable this parasite of Heliothis spp. is for development of insecticide resistant varieties. After 5 generations of inbreeding, two of five lines demonstrated an increase in LD values, going from 0.82 ug fenvalerate (in 0.5 ul acetone topical application) to a 16-fold increase at 13.12 ug with an accompanying increase in number of males being produced and a decline in line fertility. Selection ceased at the 6th round when the lines were lost due to the increased selection pressure. The observed selection response is a typical one seen for braconid wasps, and suggests this is the upper limit to which the M. croceipes genome may respond to insecticide selection. In other studies, three species of nabids were found to segregate for allozyme variation at an esterase locus (EST-1!) and an adenylate kinase locus (ADK-3). These were not in genetic equilibrium and reduction in the numbers of heterozygotes

in nature suggests strong population subdivision. These loci may serve a diagnostic function to differentiate closely related nabid species.

Publications: 88/01 to 88/12

GRASELA, J.J., STEINER, W.W.M. and MARSTON, N.L. 1988. Genetic differences at two allozyme loci in midwest populations of three species of Nabidae. Comp. Biochem. Physiol. 90B:427-431.

STEINER, W.W.M. 1988. Electrophoretic techniques for the genetic study of aphids In, A.K. Minks and P. Harrewijn (Eds.) "Aphids, Their Biology, Natural Enemies and Control", Vol. B, Elsevier Sci. Publ., Amsterdam, pp. 135-143.

18.024 CRISO034250 ANALYSIS OF THE EFFECT ON HETERCHROMATIN SEQUENCE DEFICIENT RYE CHROMOSOMES ON TRITICALE

GUSTAFSON J P; Cereal Genetics Research Unit; University of Missouri, Columbia, MISSOURI 65211.

Proj. No.: MO-8400583 Project Type: CRGD Agency ID: CRGO Period: 01 SEP 84 to 31 AUG 87

Objectives: PROJ 8400583. To ascertain the effects of the absence of blocks of telomeric heterochromatin from certain rye (Secale cereale L.) chromosomes on nucleotypic stability in wheat-rye hybrids, i.e. triticale (X Triticosecale Wittmack); and to establish the degree of nucleotypic stability of the various Secale species and of the hexaploid wheat-Secale hybrids generated with S. africanum, S. kuprijanovii, S. cereale, S. vavilovii, S. dighoricum, S. segetale, and S. dalmaticum.

Approach: To isolate triticales having rye chromosomes that are missing certain telomeric heterochromatin blocks. Analyze these triticales for any possible effects to early embryo and endosperm development of the loss of telomeric heterochromatin; and to analyze various species of Secale for early embryo and endosperm developmental patterns. Then cross all these species to a common tetraploid wheat, thus creating wheat-Secale hybrids. Analyze the wheat-Secale hybrids' early embryo and endosperm development patterns for possible nucleotypic influences coming from the various Secale species.

Progress: 87/01 to 87/12. There were three projects listed in Grant 84-CRCR-1-1412. The first project analyzed whether or not the removal of more than two blocks of rye telomeric heterochromatin resulted in further additive improvements in early seed development as was earlier suggested. The results indicated that when three rye chromosomes were present (in various combinations) there was no further additive improvement in early seed development (Dille and Gustafson, in press). The second project analyzed the possible contributions that the various species of the genus Secale could make to early seed developmental patterns. Significant variation was observed between all taxa. A significant correlation

between aberrant endosperm nuclei production and heterochromatin was observed (Gustafson and Lukaszewski, 1985a). The third project was designed to establish the effects of the Secale species developmental patterns when placed into a wheat background. The results showed that the influence of Secale species heterochromatin in the hybrids was the opposite of that observed in the species. In addition, the Secale species patterns did seem to be suppressed in a wheat background (Gustafson and Lukaszewski, 1985b).

Publications: 87/01 to 87/12

GUSTAFSON, J.P. and LUKASZEWSKI, A.J. 1985a. Early seed development in the annual and perennial Secale taxa. Can. J. Genet. Cytol. 27:134-142.

GUSTAFSON, J.P. and LUKASZEWSI, A.J. 1985b. Early seed development in Triticum-Secale amphiploids. Can. J. Genet. Cytol. 27:542-548.

DILLE, J.E. and GUSTAFSON, J.P. 1988. Influence of telomeric heterochromatin loss and development of the early embryo and endosperm in triticale. J. Plant Breeding. In press.

18.025 CRISO132315
MECHANISMS DIRECTING HORMONAL AND DEVELOPMENTAL
REGULATION OF GENE EXPRESSION IN BARLEY

ROGERS J G; Washington University, St Louis, MISSOURI 63130.

Proj. No.: MOR-8700423 Project Type: CRG0 Agency ID: CRG0 Period: 01 JUL 87 to 30 SEP 89

Objectives: To characterize fully the mechanisms by which the expression of barley alpha-amylase genes is developmentally and hormonally controlled. PROJ. 8700423.

Approach: The genes, or their promoter regions will be utilized in expression experiments in various types of cells to identify the DNA sequences responsible for limiting expression predominantly to aleurone tissue, and, appropriate changes in mRNA abundance in response to the phytohormones abscisic acid and gibberellic acid.

Progress: 88/01 to 88/12. A detailed analysis of the regulation of expression of barley alpha-amylase genes has been provided. This work showed that RNA complementary to alpha-amylase mRNA is expressed in developing barley grain and in aleurone tissue that had not been treated with hormones, or after treatment with abscisic acid. The pattern of expression of this antisense RNA suggests that it theoretically could affect expression of alpha-amylase genes. A comparison of the relative steady-state mRNA levels from individual alpha-amylase genes based on \$1 nuclease protection assays using probes from the 5' ends of alpah-amylase genomic clones demonstrated that the low-pl alpha-amylase genes are expressed at levels 5-fold greater than that of the most highly expressed high-pl alpha-amylase gene. Within the high-pl alpha-amylase gene family, steady-state mRNA levels varied by a factor of at least 10. Comparison of promoter/upstream regions from

high-level and low-level high-pl genes identified a block of nucleotides that may be associated with high-level expression. An unexpected finding was that protease and alpha-amylase genes that are unrelated other than having in common a pattern of tight hormonal regulation in aleurone tissue all utilize only a single poly(A) addition signal and their transcripts undergo 3' processing at sites centered around a common AGGCA nucleotide sequence. This pentanucleotide is followed downstream by sequences homologous to plant viroid self-cleaving RNA sequences.

Publications: 88/01 to 88/12

ROGERS, J.C. 1988. RNA complementary to alpha-amylase mRNA in barley. Plant Molecular Biology 11, 125-138.

KHURSHEED, B. and ROGERS, J.C. 1988. Barley a-amylase genes: Quantitative comparison of steady-state mRNA levels from individual members of the two different families expressed in aleurone cells. J. Biol. Chem.. in press.

18.026 CRISO096580 MECHANISMS DIRECTING HORMONAL AND DEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY

ROGERS J C; Research Office Campus Box 1054; Washington University, St Louis, **MISSOURI** 63130.

Proj. No.: MOR-8502383 Project Type: CRGD Agency ID: CRGO Period: O1 AUG 85 to O1 AUG 87

Objectives: Proj 8502383. The DNA sequence of the amylase and Alf protease/amylase inhibitor genomic clones, including the flanking regions, will be determined. A complete library of amylase genomic clones will be obtained using a vector capable of growing in a recA host, and these will be fully characterized.

Approach: The precise sequence of the amylase and Alf opposite strand transcripts, and, thereby, the sequence of their putative protein products, will be determined. This will necessitate detailed S1 nuclease mapping as well as actual cloning of the opposite strand cDNAs from appropriate tissue RNA preparations.

Progress: 87/01 to 87/08. Sequence the amylase, thiol protease, and PAPI genomic clones: The complete nucleotide sequences of the Amy32b and aleurain genomic clones have been determined, and conserved sequences that might represent sites for interaction of regulatory elements have been identified. The lambda genomic clone carrying sequences that hybridized to our PAPI cDNA probe proved to be either unstable or an artefact. We have therefore begun to screen our new library for that gene. Construct a new bacteriophage lambda library in a host deficient in recombination functions and obtain a complete set of amylase genes: Bushra Khursheed, a graduate student in my laboratory, established a library of 9 million recombinants (in toto) using our previous phage vector, lambda MG14, and a recA, recBC, sbcB host. She has isolated three new high pI amylase genes, completely sequenced one (which corresponds to our previously

characterized high pI cDNA, pM/C), and has partially sequenced another. She is in the process of using these, as well as our other genomic clones, as probes to quantitate levels of transcripts from each individual gene. Characterize the amylase opposite strand RNA: A manuscript describing characterization of the RNA complementary to amylase mRNA in barley aleurone has been submitted for publication.

Publications: 87/01 to 87/08

ROGERS, J.C., DEAN, D. and HECK, G. Aleurain: A barley thiol protease closely related to cathepsin H. (1985) Proc. Natl. Acad. Sci. USA 82, 6512-6516.

MUNDY, J. and ROGERS, J.C. Expression of a probable amylase/protease inhibitor at high levels in barley aleurone cells: comparison to the amylase/subtilisin inhibitor. (1986) Planta 169, 51-63.

WHITTIER, R.F., DEAN, D.A. and ROGERS, J.C. Nucleotide sequeuce analysis of alpha-amylase and thiol protease genes that are hormonally regulated in barley aleurone cells. (1987) Nucleic Acids Res. 15, 2515-2535.

18.027 BARLEY BREEDING AND GENETICS

CRISO093505

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BLAKE T K; Plant & Soil Sciences; Montana State University, Bozeman, MONTANA 59717. Proj. No.: MONBOO342 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 89 to 30 SEP 94

Objectives: To determine the genetics underlying water use efficiency in a defined germplasm base in barley (Hordeum vulgare L.), to identify linkages between genes modifying components of the phenotype and cloned, characterized RFLP loci. To utilize these RFLP loci in selection experiments to move genes conferring modified water use efficiency into novel genetic backgrounds.

Approach: Seventeen RFLP loci have been mapped by our laboratory in cultivated barley. We expect to have 100 mapped clones available by the end of 1990. We are monitoring the field performance of recombinant inbred lines for canopy temperature, carbon isotope discrimination, osmotic potential and yield components to determine their breeding value for each of these phenotypic characters. We will then determine associations between mapped RFLP loci and genes with significant effects on these phenotypes.

Progress: 88/01 to 88/12. The MSU Barley Breeding and Genetics Project has accomplished several of previously stated goals in 1988. We released 16 mapped genomic and cDNA clones which identify intercultivar variation and performed our first QTL analysis. In order to improve our technical capabilities, we performed a chloroplast genome mapping experiment involving alfalfa chloroplast mutants. We successfully characterized nucleotide level mutations associated with albino and chlorina phenotypes and described the physiological basis underlying these phenotypes.

Publications: 88/01 to 88/12

BLAKE, T.K. 1987. Strategies for restriction fragment length polymorphism analysis in barley. In Barley Genetics V:503-508.

BLAKE, T.K., SHIN, J.S. and SANCHEZ, M. 1989.
Development of barley RFLP Map. In
Development and application of molecular
markers to problems in plant genetics.

Current Communications in Molecular Biology. Cold Spring Harbor, NY.

LEE, D.J., BLAKE, T.K. and SMITH, S.E. 1988. Biparental inheritance of chloroplast DNA and the existence of heteroplasmic cells in alfalfa. Theor. Appl. Genet. 26(8):1-5.

LEE, D.J., BLAKE, T.K., SMITH, S.E., BINGHAM, E.T. and CARRDLL, T.W. 1989. Chloroplast genome mapping and plastid ultrastructure analysis of chloroplast deficient mutants of alfalfa. Crop Sci. 29:190-196.

18.028 CRISO034258 USE OF SINGLE COPY CLONED DNA SEQUENCES AS GENETIC MARKERS IN BARLEY

BLAKE T K; HOCKETT E A; Plant & Soil Sciences; Montana State University, Bozeman, MONTANA 59717.

Proj. No.: MDNB00343 Project Type: CRG0 Agency ID: CRGD Period: 15 SEP 84 to 31 DEC 87

Objectives: PRDJ 8400567. Dbtain cDNA derived probes from barley (H. vulgare, L.) or related angiosperms and use these to identify restriction fragment length polymorphisms in different barley genotypes. Develop these and other genomic single copy DNA derived probes using the blotting methodology of Southern (1975) to a point at which useful recombination analyses may be performed. Dur goal is to cover the barley genome with at least 100 probes which identify restriction fragment length polymophisms within commonly used genotypes of H. vulgare.

Approach: We will use the wheat-barley addition lines to locate probe hybridization sites to individual barley chromosomes. We will then test probes for frequency of restriction fragment length polymorphism identification by performing Southern blots against a diverse array of barley genotypes. Using F(2) and F(3) populations already available in our breeding program we will develop initial linkage maps based on recombination frequency.

Progress: 87/01 to 87/12. In 1987 our project identified 30 clones which identify variation among two parents used in our mapping study. These 30 clones identify simply characterized allelic variation and, to date, we have mapped 11 of them in this cross. We have 100 F2 families from individual F2 plants developed by the cross, Apex \times MMS. These F2 families have also been characterized for segregation at 10 morphological marker loci, 4 isozyme loci and 2 storage protein loci. Our fist paper describing the release of the 100 mapping lines, and a second describing the first 11 mapped clones and their release, are now being written. The first paper describing the mapping of barley chromosome 2 using morphological and isozyme markers has been

accepted with revision to Biochemical Genetics. Two further publications describing the mapping and mutation characterization of paternally transmitted mutations in the alfalfa chloroplast genome have been submitted to Crop Sci. and Theor. Appl. Genet.

Publications: 87/01 to 87/12
ND PUBLICATIONS REPORTED THIS PERIOD.

18.029* CRISO097028 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY

SANDS D C; BLAKE T K; Plant Pathology; Montana State University, Bozeman, MONTANA 59717.

Proj. No.: MDNBOO231 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 90

Objectives: To isolate and characterize genes in plants controlling development, metabolism and resistance to pathogens. To isolate and characterize genes in beneficial and pathogenic microbes controlling biological control of weeds.

Approach: Using wheat, barley and Sclerotinia-genetic systems will be studied using cell culture, mutagenesis, and DNA methods including recombinant libraries of DNA and their analysis with radioactive probes of cDNA. Restriction mapping will also be used in restriction fragment length polymorphism studies of barley. The barley - Xanthomonas host parasite system will be investigated with these methods. Host range reduction of Sclerotinia will be attempted, for applications in biocontrol of weeds.

Progress: 88/01 to 88/12. Several mutants of Sclerotinia sclerotiorum have been obtained by ultraviolet light-8-methoxy-psoralin treatment. These mutants are restricted in host range, or are auxotrophic, or are sclerotialess. In each case they may be of value as weed pathogens because of their limitation in terms of spread beyond the target host. In the past year we have found that protoplasts can be made, and regenerated from this fungus, and that their nuclear number ranges from one to more than ten. Uninucleate protoplasts would be useful for mutation experiments where auxotrophs and other recessive characteristics are desired.

Publications: 88/01 to 88/12
ZIDACK, N. K., FDRD, E., HENSON, J. and SANDS, D. C. 1988. Uninucleate protoplasts of Sclerotinia sclerotiorum for genetic manipulation. APS Abstracts 297.

18.030* CRIS0091503 RECOMBINANT DNA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY

OLESON A E; BERRYHILL D L; KOFOID K D; Biochemistry; North Dakota State University, Fargo, **NORTH DAKOTA** 58105.

Proj. No.: NDO1228 Project Type: HATCH Agency ID: CSRS Period: O1 NDV 83 to 30 SEP 88 Objectives: Clone stress response genes from selected plant species and determine the nucleotide sequences of their polypeptide reading frames and flanking regulatory regions. Elucidate the dynamics of expression of stress response genes under various stress conditions by use of recombinant DNA probes. Clone and determine the sequence of nuclear genes for ribosomal RNA from higher plants. Assess mitochondrial diversity in wheat with recombinant DNA techniques. Determine the effects on wheat mitochondrial DNA of growth in tissue culture and regeneration.

Approach: A generalized stress-response plant enzyme (RNase I) will be purified, partially sequenced (Edman method), and a synthetic oligonucleotide probe prepared. Clones from cDNA and genomic libraries will be isolated, sequenced, and used as probes of specific mRNA levels in stressed plants. A previously cloned maize rRNA gene in a lambda vector will be subcloned into plasmid and M13 phage vectors, and sequenced by the dideoxy method. Mitochondrial DNA will be isolated from several species of Triticum and Aegilops and restriction maps prepared. Sequence homologies will be determined by blotting procedures. Restriction maps of mitochondrial DNA from a single species subjected to tissue culture and regeneration will also be compared.

Progress: 83/11 to 88/09. Mitochondrial DNA has been isolated from five wheat cultivars. Each has an AABBDD nucleus, but the cytoplasmic genomes came from Aegilops squarrosa, Haynaldia villosa, T. aestivum, T. timopheevi, and T. turgidum. The DNA preparations were examined with restriction endonucleases BamHI, EcoRI, HindIII, and XhoI. Labeled probes used for this analysis were from T. aestivum or sorghum mitochondrial DNA. The results indicated that Haynaldia villosa was the B genome donor to T. turgidum and T. aestivum. In other work, a lambda clone of the nuclear ribosomal gene region of maize has been subcloned into Escherichia coli plasmid vectors. Shotgun fragments were cloned into a phage vector, and these were sequenced by the dideoxy method. This gene family is present as tandem repeats on the chromosome. The transcript encodes, in a 5' to 3' direction, 17S, 5.8S, and 26S ribosomal RNAs, with internal transcribed spacers 1 and 2 flanking the 5.8S region. Clones containing the 5.8S and spacer regions were used as probes for RFLP analysis of corn DNA. These studies indicated that little variation exists in the internal spacer regions of the multiple copies of this gene family. Sequencing of the 26S region was completed. Comparison of the alpha-sarcin domain of this RNA indicated that the 14-base core was the same as that of all other eukaryotes tested. The base flanking the 5'-end of the core is C in the case of all animals tested, whereas $\mbox{\bf U}$ is present at this position in corn, yeast and rice.

Publications: 83/11 to 88/09
MESSING, J.; CARLSON, J.; HAGEN, G.;
RUBENSTEIN, I.; and OLESON, A. 1984.
Cloning and sequencing of the ribosomal RNA
genes in maize: The 17S region. DNA
3:31-40.

18.031* CRISO096812
TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC
GENES

DUYSEN M E; Botany; North Dakota State
University, Fargo, NORTH DAKOTA 58105.
Proj. No.: NDO1924 Project Type: HATCH
Agency ID: CSRS Period: 01 NOV 85 to 30 SEP 90

Objectives: To evaluate the use of protoplasts derived from anther microspores and immature zygotic embryo cultures to regenerate cereal plants and develop techniques of inserting cytoplasmic based genes into cereals by recombinant DNA techniques and organelle transplantations.

Approach: Protoplasts isolated from microspores or cells of immature embryos will be cultured in the laboratory to regenerate cereal plants. Studies will include optimizing the hormone balance, culture medium additives, and environmental conditions. Chloroplasts will be transplanted into cereal protoplasts and the latter will be regenerated to plants. Attempts will be made to insert desirable cytoplasmic genes into chloroplasts prior to transplantation to cereal protoplasts.

Progress: 87/10 to 88/09. In collaboration with Dr. Peter Westoff (Univ. Dusseldorf, BRD) 15 different cDNA fragments of the LHC gene family have been isolated from sorghum. Each of these fragments express protein products which are antigenic to the LHCII antibody derived from wheat protein. The LHC gene family is nuclear encoded and cytoplasmic translated. Each DNA fragment has been inserted into the Lambda gtll phage and each of the latter has been inserted and cloned in the E coli bacterium. The phage DNA was reisolated from the bacterium and the plant LHC fraction was ligated into the plasmid vector, bluescript. Bluescript was used to transform E coli and the DNA was examined using miniprep techniques. Five different DNA inserts of the LHC gene family, ranging from 300-1000 bp are currently being sequenced using the T7 DNA polymerase system.

Publications: 87/10 to 88/09
DUYSEN, M., J. EIDE and K. MOGEN. 1988. The
 accumulations of LHCmRNA, LHCII protein and
 plastid pigments over greening in the CD3
 wheat mutant. Plant Physiol. (suppl.) 84.

18.032* CRISOO96560
CHROMOSOMAL LOCATIONS AND EVOLUTION OF
HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE
SPECIES

HART G E; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843. Proj. No.: TEXO6751 Project Type: HATCH Agency ID: CSRS Period: O7 AUG 85 to 30 AUG 90

Objectives: To determine the chromosomal locations of a large number of homologous gene loci in Triticum aestivum cv. 'Chinese Spring' and in seven diploid Triticeae species, to determine the genetic map positions of a selected group of these loci in Chinese Spring

and in three diploid species, and to use the findings obtained to define homoeologous relationships among individual Triticeae chromosomes and chromosome arms.

Approach: Two classes of gene loci will be studied, namely, loci which encode enzymes and loci that hybridize to unique sequence DNA probes. The chromosomal locations of Chinese Spring genes will be determined by study of compensating nullisomic-tetrasomic strains and ditelosomic strains. In diploid species, gene locations will be determined by study of wheat-alien species disomic and ditelosomic chromosome addition lines. Gene-centromere genetic distances will be determined in hexaploid wheat by the telocentric method and map distances between genes in hexaploid wheat and diploid species by Mendelian methods.

Progress: 88/01 to 88/12. The objectives of this project are to identify and determine the chromosomal locations and genetic map positions of homologous unique-sequece genetic loci in species in the tribe Triticeae and to investigate the genetic organization and evolutionary relationships among Triticeae chromosomes and genomes. Genes that encode enzymes have been studied for several years. Loci defined by the study of restriction-fragment-length polymorphisms (RFLPs) are now receiving major emphasis. Thirty-five DNA probes that definitively identify loci and another 30 probes that probably identify loci were isolated this year. About 100 RFLP loci were localized in chromosomes in Triticum aestivum cv. Chinese Spring. Several allelic variants at RFLP loci were identified. Southern blots containing genomic DNA from wheat-alien species chromosome addition lines were prepared and are now being analyzed to localize RFLP loci in alien chromosomes. Development of a set of wheat-T. searsii dosomic chromosome addition lines was completed and several ditelosomic lines and substitution lines developed. Several genes were localized in chromosomes in Haynaldia villosa.

Publications: 88/01 to 88/12

BENEDETTELLI, S. and HART, G.E. 1988. Genetic Analysis of Triticeae Shikimate

Dehydrogenase. Biochem. Genet. 26:287-301. TULEEN, N.A. and HART, G.E. 1988. Isolation and Characterization of Wheat-Elytrigia elongata Chromosome 3E and 5E Addition and Substitution Lines. Genome 30:289-292.

WHELAN, E.D.P. and HART, G.E. 1988. A
Spontaneous Translocation that Transfers
Wheat Curl Mite Resistance from Decaploid
Agropyron Elongatum to Common Wheat. Genome
30:289-292.

DEPACE, C., BENEDETTELLI, S., QUALSET, C.O., HART, G.E., SCARASCIA MUGNOZZA, G.T., VELRE, V. and VITTORI, D. 1988. Biochemical Markers in Triticum x Dasypyrum Amphiploids and Derrived Disomic Addition Lines.

DEVEY, M.E. and HART, G.E. 1988. Chromosomal Localization of RFLP Loci in Hexaploid Wheat. Proceedings of the Banbury Center Conference on Development and Application of Molecular Markers to Problems in Plant Genetics.

HART, G.E. and GALE, M.D. 1988. Guidelines for Nomenclature of Biochemical/Molecular Loci in Wheat and Related Species. Proc. 7th Int Wheat Genetics Symp. (In press). PIETRO, M.E., TULEEN, N.A. and HART, G.E. 1988. Development of Wheat-Triticum searsii Disomic Chromosome Addition Lines. Proc. 7th Int. Wheat Genetics Symp. (In press).

18.033 CRISO034382 USE OF TRANSPOSABLE ELEMENTS AND RFLP MAPPING TO CLONE QUANTITATIVE TRAIT LOCI

SHATTUCK-EIDENS D; Native Plants Inc.; 417
Wakara Way, Salt Lake City, **UTAH** 84108.
Proj. No.: UTAK-8700811 Project Type: SMALL
BUSINESS GRANT
Agency ID: SBIR Period: 01 SEP 87 to 29 FEB 88

Objectives: This proposed research represents a unique combination of technologies and subsequent opportunity to examine the molecular basis of traits exhibiting quantitative expression and inheritance. It has been observed that there are often strong associations between patterns of variation of isozyme markers and plant morphological characters which show quantitative inheritance. Recently, Helentjaris, Edwards, and Stuber (unpublished data) have extended this type of analysis using restriction fragment length polymorphisms (RFLP). Several RFLP loci were identified which were predictive for plant height.

Approach: The dwarf locus, d3, maps in one of the areas of the genome identified as significantly contributing to plant height. We propose to take advantage of this easily recognizable phenotype to clone a dwarf allele using the strategy of insertion mutagenesis with a transposable element. The application of RFLP analysis to quantitative traits, specifically plant height, has initiated the examination of this phenomenon at the DNA level. Extending the analysis by cloning through transposable element tagging will allow direct examination of gene expression.

18.034 CRISO130981 MOLECULAR AND GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY

KLEINHOFS A; Agronomy & Soils; Washington State University, Pullman, WASHINGTON 99164. Proj. No.: WNPO4745 Project Type: CRGO Agency ID: CRGO Period: O1 JUL 86 to 30 JUN 90

Objectives: PROJECT 8600397. Molecular characterization of nitrate reductase gene structure and organization. Molecular characterization of nitrate reductase-deficient mutants. Induction of nitrate reductase by light and nitrate will be investigated at the mRNA level. Nitrate reductase-deficient mutants will be induced, selected and characterized.

Approach: Nitrate reductase gene structure and mutants will be characterized by cloning, Southern blotting and sequencing. New nitrate reductase-deficient mutants will be selected by screening for chlorate resistance.

Progress: 88/01 to 88/12. A nearly full length barley NADH-nitrate reductase (NR) cDNA clone (bNRp30) was isolated and sequenced. Homology analyses indicated strong sequence conservation between barley and rice (83%), tobacco (68%), Acrabidopsis (62%) nitrate reductases. Several barley NR genomic clones were isolated. These have been partially characterized and are being sequenced.

Publications: 88/01 to 88/12

KLEINHOFS, A., WARNER, R.L., HAMAT, H.B., JURICEK, M., HUANG, C. and SCHNORR, K. 1988. Molecular genetics of barley and rice nitrate reductases. Current Topics in Plant Biochemistry and Physiology 7 (in press).

KLEINHOFS, A., WARNER, R.L., LAWRENCE, J.M., MELZER, J.M., JETER, J.M. and KUDRNA, D.A. Molecular genetics of nitrate reductase in barley. In: John L.

Wray and James R. Kinghorn, eds.

KLEINHOFS, A., WARNER, R.L. And MELZER, J.M. Genetics and molecular biology of higher plant nitrate reductases. In: Recent Advances in Phytochemistry (Proc.

Int'l. Symposium, Plant Nitrogen Metabolism, Iowa City, 1988)(in press).

18.035 CRISO141359
PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR
REGULA- TION OF OAT STORAGE PROTEINS

PETERSON D M; Agricultural Research Service, Madison, ${\it WISCONSIN}~53702$.

Proj. No.: 3655-21230-001-00D

Project Type: INHOUSE Agency ID: ARS Period: 31 MAR 86 to 31 MAR 89

Objectives: Determine hormonal effects on synthesis and accumulation of storage proteins in oat endosperm. Identify high-protein germplasm in oats. Developand adapt biochemical and molecular methods for oat cultivar identifica- tion.

Approach: Cytokinins and abscisic acid (ABA) will be applied to oat plants and cultured spikelets and effects on storage proteins measured. Endogenous hormone levels will be measured immunologically and correlated with rates of protein synthesis. Breeders' samples will be analyzed for protein and moisture by near infrared reflectance spectroscopy. Oat cultivars will be characterized by electrophoresis of their storage proteins. The feasi-lity of immunological techniques and restriction fragment length polymorp-hisms for cultivar identification will be investigated.

Progress: 88/01 to 88/12. A culture system was devised where oats in the growth chamber were subjected to decreasing supplies of inorganic sulfur in the nutrient media, which resulted in plants producing seed that contained about 50% of the normal concentration of sulfur. Analysis of storage protein

fractions by Western blotting and by solvent fractionation revealed that the low-sulfur plants were depleted of avenins which are relatively sulfur-rich, but globulins which are sulfur-poor were less affected. cDNA probes for avenin and globulin genes were obtained, and will be used to measure quantities of mRNA for these protein fractions as affected by sulfure status. During 1988, 6853 samples of oat groats from plant breeders in seven states were analyzed for protein by NIR reflectance, and an additional 2046 by Kjeldahl for a total of 8899 samples.

Publications: 88/01 to 88/12
 PETERSON, D.M., DAILEY, J.M., and OSBORN,
 T.C. 1987. Regulation of hordein synthesis
 in a low-protein barley cultivar. IN Barley
 Genetics V, Proc. Fifth Int. Barley Genet.
 Symp., p. 509-514, Okayama, Japan.

DAILEY, J.M., PETERSON, D.M., and OSBORN, T.C. 1988. Hordein gene expression in a low protein barley cultivar. Plant Physiol. 88:450-453.

WELCH, R.W., PETERSON, D.M., and SCHRAMKA, B. 1988. Hypocholesterolemic and gastrointestinal effects of oat bran fractions in chicks. Nutr. Rep. Int. 38:551-561.

PETERSON, D.M., FORDE, J., WILLIAMSON, M.S., ROHDE, W., and KRIES, M. 1988. Nucleotide sequence of a barley genomic clone for chymotrypsin inhibitor-2. J. Cell Biochem. Suppl. 12C:184.

18.036 CRISO096015
THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION
AND EXPRESSION

QUAIL P H; Botany; University of Wisconsin, Madison, **WISCONSIN** 53706.

Proj. No.: WIS-8500295 Project Type: CRG0 Agency ID: CRG0 Period: 01 SEP 85 to 31 AUG 87

Objectives: Proj 8500295. The long-term goal of this research is to understand the molecular mechanism by which phytochrome regulates plant development in response to light. Specific objectives are to define structural properties of the phytochrome molecule potentially responsible for its regulatory function and to understand the molecular basis for the photoreceptor's control of gene expression.

Approach: Sequencing of Avena cDNA clones to deduce the entire amino acid sequence of the polypeptide; sequencing of Avena phytochrome genes and flanking regions as a step toward identifying sequences involved in regulating their transcription; sequencing of phytochrome clones from a dicotyledon in order to identify evolutionarily conserved structural features.

CM 20 FORAGE CROPS

20.001 CRISO099288
SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN
MEDICAGO

MCCOY T J; Plant Science; University of Arizona, Tucson, **ARIZONA** 85721.
Proj. No.: ARZT-385350-G-25 Project Type: CRGO Agency ID: CRGO Period: O1 AUG 86 to 31 JUL 89

Objectives: PROJECT 8600513. To determine genomic affinities of Medicago species. The long-term goal is to utilize knowledge obtained to attempt the synthesis of chromosomally stable, hexaploid populations of alfalfa by the incorporation of a homoeologous genome from another Medicago species resulting in a hexaploid with two genomes from another Medicago and four genomes from M. sativa.

Approach: Produce triploid (2n=3x=24) interspecific hybrids between M. sativa and M. papillosa, M. sativa and M. chodopea and M. sativa and M. rupestris. Detailed cytogenetic analysis of these hybrids will be conducted to determine genomic affinities. Promising triploid interspecific hybrids will be chromosomally doubled to produce hexaploids of novel genomic constitution. Hexaploids will be studied in detail to determine chromosome stability. In addition preliminary evaluation of fertility and yield will be conducted.

Progress: 88/01 to 88/12. Medicago sativa x M. papillosa triploid hybrids containing one genome of M. sativa and two genomes of M. papillosa were chromosomally doubled to produce alloautohexaploids (2n = 6x = 48) with two genomes of M. sativa and four genomes of M. papillosa, designated SSPPPP. Seven SSPPPP hexaploids were intercrossed. Chromosome counts of 47 progeny from intercrossing the SSPPPP hexaploids demonstrated all were hexaploid, indicating chromosome stability. Chromosome stability of these is important because autohexapoloids of M. sativa are chromosomally unstable. This M. sativa autohexaploids cannot be used commercially even though autohexaploids outyield autotetraploids. Designing novel genomic combinations based on genomic affinity may result in higher yielding alfalfa populations that have a modified cytogenetic structure. We are using trisomic progeny (2n = 2x + 1 = 17) from backcrossing triploid M. sativa \times M. papillosa hybrids to $2\times$ M. papillosa. The extra chromosome in the trisomic progeny is an intact M. sativa chromosome in addition to the two complete sets of M. papillosa chromosomes. Therefore, an allozyme or RFLP unique to the M. sativa parent (that produced the SPP triploid) that appears in the trisomic maps to the extra chromosome from M. sativa. This procedure has been used to map the Lap 1 locus to chromosome 8, and to demonstrate that chromosome 8 is not the location of Lap 2. Prx 1 and Prx 2.

Publications: 88/01 to 88/12
MCCOY, T. J. and BINGHAM, E. T. (1988).
 Cytology and cytogenetics of alfalfa.
In: Alfalfa and alfalfa improvement. Edited
 by: Hanson, A. A., Barnes, D. K., and Hill,
 R. R. Jr. American Society of Agronomy,
 Madison, WI. Monograph 29:737-776.

20.002 CRISO131832
CYTOGENETIC MANIPULATIONS FOR ALFALFA
IMPROVEMENT

MCCOY T J; Plant Science; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-136306-H-25-031

Project Type: HATCH Agency ID: CSRS Period: O1 JUL 87 to 30 SEP 90

Objectives: Synthesize and evaluate new cytogenetic combinations in alfalfa; and to test the potential of reproductive mutants in alfalfa improvement.

Approach: Cytogenetic, biochemical and morphological studies will be conducted on new Medicago interspecific hybrid combinations. All F(1) hybrids will be tested for crossability in backcross to alfalfa to determine whether useful traits can eventually be transferred from the wild species to cultivated alfalfa. In addition, in-depth cytogenetic analysis will be conducted to determine the genomic affinities between cultivated alfalfa and wild Medicago species. In addition to conventional cytogenetic analysis, molecular markers including isozymes and restriction fragment length polymorphisms RFLPs will be used to confirm the absence or presence of genetic recombination between wild and cultivated genomes.

Progress: 88/01 to 88/12. Recovering interspecific hybrids between alfalfa, Medicago sativa L. and other species of the subgenus Medicago has opened new avenues for genetic manipulation of alfalfa. In addition to the potential for introgressing economic traits (e.g., disease resistance, insect resistance and stress tolerance) from the wild species into cultivated alfalfa, the wild species offer the possibility of ultimately improving yield per se. Cytogenetic and genetic analyses of interspecific hybrids between alfalfa, Medicago sativa L. and other Medicago species were conducted to determine genomic affinities. Tetraploid hybrids containing two genomes of M. sativa and two genomes of either M. rhodopea Velen. or M. rupestris M.B. were produced by chromosome doubling the diploid interspecific hybrid (recovered from ovule-embryo cultures). Cytogenetic analysis of diploid, triploid and tetraploid hybrids indicated a lack of genomic affinity between M. sativa and M. rhodopea and M. sativa and M. rupestris. Disomic inheritance of two loci for lucine-aminopeptidase, Lap 1 and Lap 2, and two loci for peroxidase, Prx 1 and Prx 2 was observed in the progeny from crossing the tetraploid hybrids. This provides evidence of an allotetraploid cytogenetic structure of the tetraploid hybrids. Because of the apparent absence of genetic recombination between M. sativa and either M. rhodopea or M. rupestris genomes, it will be difficult to introgress genes from these into alfalfa.

Publications: 88/01 to 88/12
MCCOY, T.J. (1988). Tissue culture selection
 for disease resistant plants. Iowa State J.
 Res. 62:503-521.
CHAULK, C.A. and MCCOY, T. J. (1988).
 Chromosome number fertility and
 mitochondrial genomes of backcross

populations derived from Medicago sativa x M. dzhawakhetica hybrids. Agronomy Abstracts, pg. 77.

20.003 CRISO068563 INHERITANCE OF MITOCHONDRIAL DNA IN SOMACLONAL VARIANTS

MCDANIEL R G; Plant Science; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-136248-H-25-O23

Project Type: HATCH Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 88

Objectives: Comparative evaluations of possible mitochondrial DNA variation in a series of alfalfa somaclones and germplasm sources. The possible correlation between plant vigor and productivity and specific base sequence comparison of mitochondrial DNA from somaclones will be investigated.

Approach: A series of alfalfa somaclonal variants will be produced by in vitro tissue culture techniques. Regenerated somaclonal variants will be used as a source of mitochondrial DNA. Purified mitochondrial DNA will be assessed for genotypic differences using appropriate gel electrophoresis restriction endonuclease fragment analysis.

Progress: 75/07 to 88/09. A series of alfalfa somaclones regenerated from tissue culture have been characterized on the basis of several physiological traits including esterase isozyme profile and mitochondrial respiratory efficiency. Selfed seed is being utilized to establish a base population as a control for crosses of these somaclones now being made. These physiologically distinct parents are constructed for use in experiments testing possible diversity of mitochondrial DNA, judged by restriction fragment length polymorphisms. Cluster analysis software developed for this project is designed to be utilized to determine genetic relationships among somaclonal variants and their cross progeny. Such experiments should enable a comparison between mt DNA evolutionary rates and changes in those physiological traits partially under control of mt DNA. TERMINATED 30 SEP 88.

Publications: 75/07 to 88/09
BAERTLEIN, D.A. (1988). Genetics of mineral
phosphate solubilization (MPS) in
Escherichia coli. Ph.D. Thesis 99 p.
University of Arizona.

20.004* CRISO137480 BARLEY GENETICS AND PLANT CYTOGENETICS

TSUCHIYA T; HANG A; WANG S; Agronomy; Colorado State University, Fort Collins, **COLORADO** 80523.

Proj. No.: COLOO625 Project Type: HATCH Agency ID: CSRS Period: 14 FEB 89 to 30 JUN 93

Objectives: The overall objective of this project is basic genetic and cytogenetic studies in various plant species for aiding the progress in genetics and their direct and/or indirect uses in plant breeding programs.

Approach: We use chromosome manipulation approaches in most of the research work. For barley genetics chromosomal mutants, mainly various types of trisomics and many genetic mutants are used to improve genetic linkage maps and study the genetic architecture of barley chromosomes. For other materials, karyotype analysis by conventional and/or Giemsa-banding techniques of chromosome studies will be used.

20.005 CRISO096366 ORGANELLE DNA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM

SMITH R L; PRING D R; Agronomy; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-AGR-02513 Project Type: CRG0 Agency ID: CRG0 Period: 01 JUL 85 to 30 SEP 87

Objectives: Proj 8500311. Study genetic mechanisms of cytoplasmic male sterility (CMS) on the molecular level, study mitochondrial and chloroplast genome organization and evaluate maize DNA probes for locating and characterizing several mitochondrial genes in Pennisetum americanum.

Approach: Compare organelle DNA isolated from several sterile cytoplasms to fertile counterparts (B lines) using endonuclease restriction enzyme analyses and Southern blots. Also compare fertile revertants to their CMS parentals and CMS mutants will be compared to their fertile parentals. Sequences that differ in CMS-fertile comparisons will be cloned and characterized in detail. Homology of maize and sorghum sequences believed to be associated with CMS and identified as protein coding genes will be evaluated in Pennisetum.

Progress: 87/10 to 88/06. Mitochondrial DNA restriction endonuclease fragment patterns were evaluated for classifying cytoplasmic male sterile and fertile cytoplasms and the relative effectiveness of restriction fragment and maize mitochondrial gene hybridization patterns to distinguish the cytoplasms was determined. BamHI restriction patterns differentiated the male sterile cytoplasms, S-Am, S-A1, S-A3, but grouped together S-A2 and the five male sterile mutants derived from F-B2. The CMS mutant, S-M7, had a unique SmaI restriction pattern differentiating it from S-A2 and the other four CMS mutants and verifying its mutant origin. Hybridization patterns, using maize mitochondrial gene probes, differentiated the cytoplasms much the same as did the restriction patterns. Unique restriction fragments and patterns capable of differentiating the various cytoplasms were identified. Comparisons of the mtDNA of the CMS mutants to that of their fertile parental, Tift 239DB, and to that of their fertile revertants identified DNA

fragments that were rearranged by mutation and reversion. Hybridization with maize mitochondrial gene probes indicated that those rearrangd fragments were associated with the atp6 gene. Mutation from fertile to CMS was associated with the gain of a atp6-hybridizing 7.3 kb BamHI fragment and a loss of that fragment upon reversion back to fertility. However, observations of other rearranged fragments indicated that revision was not the reversal of the mutation to CMS.

Publications: 87/10 to 88/06 SMITH, R.L. and CHDWDHURY, M.K.U. 1989. Mitochondrial DNA polymorphism in male sterile and fertile cytoplasms in pearl millet. Crop Sci. (in press). SMITH, R.L. and CHDWDHURY, M.K.U. 1988. Mitochondrial genomic rearrangements in cytoplasmic male sterile mutants and their fertile revertants. International Society for Plant Molecular Biology.

20.006 CRISO131969 GENE AMPLIFICATION TO IMPROVE NITROGEN ASSIMILATION AND BIOMASS YIELD OF NAPIERGRASS

SCHMIDT R R; Microbiology & Cell Science; University of Florida, Gainesville, FLORIDA 32611.

Proj. No.: FLA-MCS-02653 Project Type: STATE Agency ID: SAES Period: 01 JUL 86 to 30 JUN 91

Objectives: The long-range objective is to decrease nitrogen-fertilizer costs for production of biomass from Napiergrass so that its use as a feedstock for microbial conversion to methane gas will be more economical on a commercial scale. The short-term objective is to attempt to increase the rate of assimilation of nitrate/ammonium by Napiergrass plants by amplifying the number of copies of the gene (i.e., gene amplification) encoding the glutamine synthetase isoenzyme involved in net assimilation of ammonium for use in biosynthesis of nucleotides and the primary amino acid, glutamate.

Approach: A combination of plant tissue culture and molecular biology procedures (including recombinant DNA technology) will be used to induce and select for Napiergrass variant cell-lines that have elevated levels of the nitrogen-assimilatory glutamine synthetase due to an increased copy number of its encoding gene. The variant cell-lines will be regenerated into intact plants for measurements of rates of nitrogen assimilation and biomass yield.

Progress: 87/10 to 88/09. The objective of the research is to use a combination of molecular biology and tissue culture procedures to induce and select variants of Napiergrass (a biomass crop) that have amplified copy numbers of the genes encoding the cytosolic and chloroplastic glutamine synthetase (GS) isoenzymes (GS(1) and GS(2)), respectively. A cDNA library was prepared, in a new lambda vector (Stratagene lambda ZAP), from total poly(A) RNA isolated from Napiergrass leaves. This library was screened with two heterologous GS cDNA probes (from Phaseolus vulgaris) and 31 different Napiergrass GS cDNA clones were isolated. These Napiergrass cDNA clones have been sized, and all but one are over 1.0 kb and six are over 1.4 kb. Since GS mRNAs in legumes are known to range up to 1.6 kb, the longest Napiergrass cDNAs are possibly full-length are nearly full-length clones of their corresponding mRNAs. Restriction mapping has indicated that the longest cDNAs isolated are encoded by at least two different genes. Thus, Napiergrass cDNAs, encoding the cytosolic and chloroplastic GS mRNAs, may have been isolated. After charcterization, the two different types of cDNA clones will be used as hybridization probes to isolate Napiergrass cell-lines with amplified genes encoding GS(1) and GS(2) isoenzymes. A genomic DNA library was also prepared and is currently being screened for clones carrying the genes encoding the two GS isoenzymes.

Publications: 87/10 to 88/09 ND PUBLICATIONS REPORTED THIS PERIOD.

20.007 CRISO098433 GENE EXPRESSION IN PLANTS: A STUDY OF POLYADENYLATION IN PLANTS

HUNT A G; Agronomy; University of Kentucky, Lexington, **KENTUCKY** 40506.

Project Type: CRGD Proj. No.: KY01229 Agency ID: CRGD Period: O1 SEP 85 to 31 AUG 89

Objectives: Proj. 8502524. The objectives of this project are to define and characterize the molecular signals that direct the polyadenylation of messenger RNAs in plants.

Approach: The experimental approaches to be used involve the cloning and in vitro mutagenesis of the 3' regions of various nuclear genes from plants, subcloning the resultant mutants into a specialized test vehicle, and transfer of the chimeric genes into tobacco cells using Ti plasmid-derived transformation vectors. The ability of the mutant 3' regions to direct polyadenylation will be analyzed by Northern and S1 nuclease analysis.

Progress: 88/01 to 88/12. The RNA sequences that direct mRNA 3' end formation for transcripts from a pea gene for the small subunit of ribulose 1.5-bisphosphate carboxylase (rbcS) have been functionally mapped by deletion mutagenesis and Ti plasmid-mediated gene transfer. Sequences between 6 and 137 bases 5' of the "normal" sites of polyadenylation in the rbcS-E9 gene (bases -6 to -137) are required for mRNA 3' end formation at these sites. In the absence of these sequences, mRNAs carrying the remainder of the rbcS-E9 region (bases -6 to +411) direct mRNA 3' end formation an array of cryptic sites in this region. The region between -137 and -235 has two distinct effects on mRNA 3' end formation. When this region is removed by deletion, two classes of stable RNAs arise from test genes. One of these is polyadenylated and ends at the normal rbcS-E9 poly(A) addition sites. The other is not polyadenylated and has

3' ends near the CAT-rbcS junction in the test genes. When bases -235 to -111 are added to bases -6 to +411 (in effect creating a mutant in which bases -110 to -7 have been deleted), the utilization of an otherwise minor polyadenylation site is enhanced. Finally, there are redundant downstream elements for polyadenylation at the normal poly(A) sites. The polyadenylation signal of the cauliflower mosaic virus (CaMV) 195/35\$ transcription unit has been mapped by deletion mutagenesis. The upstream element needed for polyadenylation at the previously-reported site in the CaMV genome lies between 1 and 271 bases from this site.

Publications: 88/01 to 88/12
HUNT, A.G. (1988). Identification and
 characterization of cryptic polyadenylation
 sites in the 3' region of a pea ribulos
 1,5-bisphosphate carboxylase small subunit
 gene. DNA 7:329-336.
HUNT, A.G. and GRAYBOSCH, R. (1988).
Messenger RNA 3' end formation in
 transgenic plants. J. Cellul. Biochem.
Supplement 12D, 6O.

20.008 CRISO097154 REPEATED DNA SEQUENCES AND CHLOROPLAST DNA INSTABILITY IN CLOVER

PALMER J D; Biological Sciences; University of Michigan, Ann Arbor, **MICHIGAN** 48109. Proj. No.: MICR-8502762 Project Type: CRG0 Agency ID: CRG0 Period: 30 SEP 85 to 31 JAN 88

Objectives: Proj 8502762. The objectives of this study are to characterized in detail the structure and evolution of two unique feature of clover chloroplast DNA - its accelerated rate of sequence rearrangements and its family of dispersed repeated sequences - and to investigate where there is a direct causal relationship between the two. Each of the six or more dispersed repeat elements in the chloroplast genome of subclover, Trifolium subterraneum, will be localized on a genomic restriction map, isolated, and sequenced. Experiments will be conducted to assess possible genetic functions of these repeats. We will search for recent chloroplast DNA rearrangements and examine their endpoints in order to understand how these changes occur. Finally, we plan to investigate whether the repeats originated from within the chloroplast or from extra-chloroplastic sources, such as the mitochondrial or nuclear genomes.

Approach: The principal experimental approaches to be used are molecular cloning, DNA filter hybridization and DNA sequencing.

Progress: 85/09 to 88/01. A complete clone bank, restriction site map, and gene map for 35 genes were established for the chloroplast genome of subclover, Trifolium subterraneum. Hybridization experiments comparing genome organization in subclover to other flowering plants reveal that subclover cpDNA has undergone an unprecedentedly high level of internal rearrangement. Many inversions, deletions and transpositions have occurred to restructure gene order in subclover cpDNA.

Associated with this unusually unstable genome are two structural features potentially involved in the rearrangements. A dispersed family of repeats, with each element about 1 kb in length, is present in at least six copies. A survey of a wide taxonomic range of species indicates that these elements are unique to the cpDNAs of subclover and two closely related species. Several of the repeats are associated with genomic rearrangements and one repeat is inserted within a normally highly conserved series of ribosomal protein genes, effectively replacing a gene, rp122, that is missing from subclover cpDNA. Sequencing of the repeats provides suggestive evidence, but no proof, that they may be transposable elements. They do contain terminal direct repeats of about 300 bp, but lack significant homology to any known transposable elements or to reverse transcriptase and transposase genes.

Publications: 85/09 to 88/01 PALMER, J.D., OSORIO, B., ALDRICH, J. and THDMPSDN, W.F. 1987. Chloroplast DNA evolution among legumes: Loss of a large inverted repeat occurred prior to other sequence rearrangements. Curr. Genet. 11:275-286.

PALMER, J.D., OSORIO, B. and THOMPSON, W.F. 1988. Evolutionary Significant of inversions in legume chloroplast DNAs. Curr. Genet. 14:65-74.

MILLIGAN, B.G., HAMPTON, J. and PALMER, J.D. 1988. Dispersed repeats and structural reorganization in subclover chloroplast DNA. Molec. Biol. Evol., submitted.

MILLIGAN, B.G. 1988. Purification of chloroplast DNA using hexadecyltrimethylammonium bromide. Anal. Biochem., submitted.

MILLIGAN, B.G. and PALMER, J.D. Subclover chloroplast ribosomal protein genes: rp123 is partially duplicated and rp122 is missing. In preparation.

MILLIGAN, B.G. and PALMER, J.D. Repeated elements in the chloroplast genome of Trifolium subterraneum. In preparation.

MILLIGAN, B.G. Differentiation of chloroplast DNA within and among populations of Trifolium pratense. In preparation.

20.009* CRISO049430 GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS

STEINER W M M; Parasite-predator Bio & Ecol Bio Control of Insects Lab; Agricultural Research Service, Columbia, **MISSOURI** 65211. Proj. No.: 3622-24000-006-00D

Project Type: INHOUSE Agency ID: ARS Period: 01 OCT 84 to 30 SEP 89 (

Objectives: Genetically map and characterize chromosomal, morphological, DNA, and allozyme variants of selected entomophagous insects and determine their physiological, developmental and behavioral significance and their potential for utilization in bioengineering programs to enhance entomophageefficiency.

Approach: Populations of selected entomophages will be assayed using classical gene- tic techniques and restriction enzyme (DNA) technology to establish gene- tically typed isolines. These will be used to establish linkage maps and study genome structure in the context of predator-parasite/prey models. About 80 % of effort will be devoted to the model offered by Heliothis zea and its entomophages with 20% of effort reserved to develop genetic know-ledge of other models. Selected variants will also be biochemically char- acterized and their distributions in natural populations determined. Lab- oratory tests of physiological, developmental and behavior response under stresses posed by pesticides, temperature and desiccation will reveal whichphenotypes can enhance natural population fitness characteristics. Methods of transferring desirable traits will be developed.

Progress: 88/01 to 88/12. Selection for insecticide resistance in females of the parasitoid Microplitis croceipes was initiated to determine how amenable this parasite of Heliothis spp. is for development of insecticide resistant varieties. After 5 generations of inbreeding, two of five lines demonstrated an increase in LD values, going from 0.82 ug fenvalerate (in 0.5 ul acetone topical application) to a 16-fold increase at 13.12 ug with an accompanying increase in number of males being produced and a decline in line fertility. Selection ceased at the 6th round when the lines were lost due to the increased selection pressure. The observed selection response is a typical one seen for braconid wasps, and suggests this is the upper limit to which the M. croceipes genome may respond to insecticide selection. In other studies, three species of nabids were found to segregate for allozyme variation at an esterase locus (EST-1!) and an adenylate kinase locus (ADK-3). These were not in genetic equilibrium and reduction in the numbers of heterozygotes in nature suggests strong population subdivision. These loci may serve a diagnostic function to differentiate closely related nabid species.

Publications: 88/01 to 88/12

GRASELA, J.J., STEINER, W.W.M. and MARSTON, N.L. 1988. Genetic differences at two allozyme loci in midwest populations of three species of Nabidae. Comp. Biochem. Physiol. 90B:427-431.

STEINER, W.W.M. 1988. Electrophoretic techniques for the genetic study of aphids In, A.K. Minks and P. Harrewijn (Eds.)
"Aphids, Their Biology, Natural Enemies and Control", Vol. B, Elsevier Sci. Publ., Amsterdam, pp. 135-143.

20.010 CRISO134816 SYNTHETIC AND POSTSYNTHETIC CHANGES IN HISTONES OF ALFALFA IN ADAPTION TO SALT TOLERANCE

WATERBORG J H; Biochemistry; University of Nevada, Reno, ${\it NEVADA}$ 89557.

Proj. No.: NEVOO168 Project Type: STATE Agency ID: SAES Period: O1 MAY 88 to O1 AUG 88

Objectives: Test the hypothesis that changes in histone variant composition and postsynthetic modification may compensate for the effects of increased intranuclear ionic strength in alfalfa (Medicago sativa) tissue culture cells grown in media containing 1% NaCl. Such changes could be essential elements in the complex pattern of salt tolerance development in glycophytic plants by maintaining chromatin structure in a functional state.

Approach: Comparative analysis of histone variant composition and postsynthetic modification (acetylation and phosphorylation) by high resolution gel analysis. Steady state stain and radiolabel dynamic assessments of changes between isogenic alfalfa strains that are sensitive or tolerant to growth at 1% NaCl. Cloning and sequence analysis of the histone H3 variant genes and proteins and assessment of specific chromatin localization.

Progress: 88/05 to 88/08. Dr. Waterborg has left this institution and took grant with him.

Publications: 88/05 to 88/08 NO PUBLICATIONS REPORTED THIS PERIOD.

20.011 CRISO134989 MOLECULAR SYSTEMATICS OF THE LEGUME TRIBE PHASEOLEAE AND ALLIES

DOYLE J J; Bailey Hortorium; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-187303 Project Type: STATE Agency ID: SAES Period: 01 JUL 88 to 30 JUN 91

Objectives: The purpose of this research is to elucidate phylogenetic relationships in the large and economically important legume tribe Phaseoleae. Specific goals include: generic phylogeny of subtribes, particularly Phaseolinae, Glycininae, Cajaninae, and Diocleinae, the determination of the closest geeric relatives of such important crop plants as common bean, pigeon pea, and soybean. Establishing a subtribal phylogeny of Phaseoleae determining the placement of Phaseoleae with respect to other papilionoid legume tribes, particularly putatively primitive tropical woody groups such as Millettieae.

Approach: Numberous genera will be surveyed for a variety of molecular markers. These will include rare chloroplast DNA structural mutations, particularly a 78 kilobase inversion and the deletion of the inverted repeat, as well as a 500 base pair insertion in the genes encoding a subunit of the 75 seed storage proteins and a duplication of the gene encoding the cytosolic isozyme of glucosephosphate isomerase. These and other rare markers will be used to define groups for further study, using such approaches as restriction mapping of chloroplast genomes and sequences of specific chloroplast and nuclear genes.

20.012* CRISO137809
CELLULAR AND MOLECULAR GENETICS FOR IMPROVEMENT
OF MAIZE AND FESCUE

FERGUSON N H; Agronomy & Soils; Clemson University, Clemson, **SOUTH CAROLINA** 29634. Proj. No.: SCO1313 Project Type: HATCH Agency ID: CSRS Period: O5 APR 89 to 30 DEC 92

Objectives: To identify strains of the tall fescue endophyte Acremonium coenophialum and to correlate molecular genetic markers with observed differences in plant response. To analyze development of male and female flowers in majze.

Approach: Strains of endophyte will be identified using RFLP analysis. RFLP markers will be correlated with differences in plant response. Interaction of grass and endophyte will be examined using molecular genetic analysis. Flower development in maize will be manipulated using tissue culture technology. Molecular genetic analysis will help identify differences in genes and gene expression.

20.013* CRISO135474
GENETIC STRUCTURE OF SMALL GRAIN PATHOGEN
POPULATIONS AND MANAGEMENT OF GENETIC
RESISTANCE

MCDONALD B A; Plant Pathology & Microbiology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6928 Project Type: HATCH Agency ID: CSRS Period: 11 AUG 88 to 31 JUL 93

Objectives: 1) Monitor presence and assess severity of small grain diseases in South Texas. 2) Develop electrophoretic genetic markers to study the population genetics of fungal pathogens of small grains. 3) Obtain estimates of the distribution of genetic variability in pathogen populations in Texas. 4) Develop and test gene-management strategies that are likely to control small grain diseases. 5) Compare effects of different gene-management strategies on the genetic structure of pathogen populations. 6) Enlarge and diversify the genetic base of small grains in Texas.

Approach: Isozyme and RFLP genetic markers will be developed first for the wheat/wheat leaf rust pathosystem. These electrophoretic markers will be used to quantify genetic variation in pathogen populations in Texas and to measure the response of P. recondita populations to different gene-management strategies. Wheat containing different types and combinations of resistance genes will be deployed in pure stands and in cultivar mixtures in replicated field plots and compared for effectiveness of disease control.

Progress: 88/01 to 88/12. Knowledge of the population genetics of pathogen populations is needed to make more effective use of resistance genes. Restriction fragment length polymorphisms (RFLPs) are being developed as

tools for studying the population genetics of small grain pathogen populations. Rapid miniprep DNA extraction protocols were developed for the wheat pathogens Septoria tritici and Puccinia recondita. Genomic DAN libraries consisting of anonymous 0.5-2.2 kb fragments of S. tritici and P. recondita DNA were cloned into plasmid pGEM4. Though screening for RFLPs did not begin until December, preliminary results are promising; the first two probes tried from the S. tritici library detected RFLPs in S. tritici. P. recondita isolates will be screened shortly. These preliminary results suggest the RFLPs will provide the tools necessary for studying population genetics of pathogen populations.

Publications: 88/01 to 88/12
MCDONALD, B.A., ALLARD, R.W. and WEBSTER,
R.K. 1988. Response of two-, three-, and
four-component barley mixtures to a
variable pathogen population. Crop Sci.
28:447-452.

20.014* CRISO090986 STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS

RYAN C A; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO1791 Project Type: HATCH Agency ID: CSRS Period: O1 SEP 85 to 31 AUG 90

Objectives: The objectives of this research are to understand the biochemical and molecular biological basis of insect-induced synthesis and accumulation of proteinase inhibitor proteins in plants. The complete chemical nature of the wound signals will be sought and their mechanisms of release, transport, intracellular recognition and gene activation will be studied. The structure and organization of the inhibitor genes will be investigated and the wound-induced promoter regions characterized. These promoters will be employed to improve the natural defenses of important crop plants.

Approach: Modern biochemical, immunological and molecular biological techniques will be employed, including recombinant DNA technology.

Progress: 88/01 to 88/12. Two 5' regions of the wound-inducible potato inhibitor II gene that regulate wound-induction have been identified by deletion analysis in collaboration with Dr. Gyn An. These regions, near 650 bp and 150 bp upstream from the translation initiation codon, were shown by gel retardation assays in this laboratory to be the only regions that bind to specific tomato leaf wound-inducible nuclear proteins. One of these trans-factors has been partially purified and has a Mr of 27 kDa. The wound-inducible expression of a fused Inhibitor II-CAT gene in tobacco was shown to be enhanced over 50-fold by sucrose or other metabolizable sugars. This increase is due to transcriptional control, indicating that mRNA synthesis is somehow being regulated by a sucrose-derived molecule. A cDNA coding for wound-inducible trypsin inhibitor in

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alfalfa leaves (called ATI, a member of the Bowman-Birk inhibitor family) was isolated and characterized. The gene has also been isolated and is currently being characterized. A strongly expressed Inhibitor I gene has been isolated from the DNA of a wild species of tomato, L. peruvianum. This Inhibitor I gene is being introduced into the modern tomato where it is not expressed in fruit. The expression in modern fruit could allow the development of a fruit expression system. Oligosaccharides that induce the accumulation of proteinase inhibitors in plants have been shown to cause the enhanced phosphorylation of plasma membrane proteins from potato and tomato.

Publications: 88/01 to 88/12

CLORE, G.M., GRONEBORN, A.M., NILGES, M. and RYAN, C.A. (1988). The Three-Dimensional Structure of Potato Carboxypeptidase Inhibitor in Solution: A Study Using Nuclear Magnetic Resonance, Distance Geometry and Restrained Molecular Dynamics. RYAN, C.A. and AN, G. (1988). Molecular

Biology of Proteinase Inhibitors in Plants. Plant, Cell and Environment 11:345-349.

RYAN, C.A. (1988). Oligosaccharide Signalling for Proteinase Inhibitors in Plant Leaves. In "Advances in Phytochemistry" (Conn. E., ed.) Vol. 22, Plenum Press, NY, pp. 163-180.

PEARCE, G., LILJEGREN, O. and RYAN, C.A. (1988). Proteinase Inhibitors in Fruit of the Wild Tomato Species L. peruvianum: A possible Mechanism for Plant Protection and Seed Dispersal. Planta 175:527-531.

AN, G., THORNBURG, R.W., JOHNSON, R., HALL, G. and RYAN, C.A. (1988). A Possible Role for 3' Sequences of the Wound-Inducible Potato Proteinase Inhibitor IIK Gene in Regulating Gene Expression. In "NATO Conference Proceedings".

RYAN, C.A. (1988). Proteinase Inhibitor Genes: Strategies for Manipulation to Improve Natural Plant Defense. BioEssays (in press).

GREENBLATT, H.M., RYAN, C.A. and JAMES, M.N.G. (1988). Structure of the Complex of Streptomyces Griseus Proteinase B andPolypeptide Chymotrypsin inhibitor I from Russet Burbank Potato Tubers at 2.1: A Resolution (in Press).

20.015 CRISOO96938 ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA

RYAN C A; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNP05793 Project Type: CRG0 Agency ID: CRG0 Period: 01 SEP 85 to 31 AUG 87

Objectives: Research is proposed to prepare a library of alfalfa genes in lambda phage and isolate a wound-induced trypsin inhibitor (ATI) gene. The gene will be utilized as a model to determine the regions that are essential for the wound-regulated expression of a plant protection gene. Gene fusions of the wound-induced promoter of the ATI gene with a chloramphenicol acetyl transferase (CAT) gene

will be used to transform tobacco, tomato and alfalfa plants with the now wound-inducible CAT gene. The successful transformation into one or more of these plants will provide a sensitive assay system (the CAT enzyme) to study wound-induced promoter structure and function.

Approach: A library of alfalfa genes will be constructed and the ATI gene isolated using a Bowman-Birk gene from soybeans. The nucleotide sequence of the gene will be determined by conventional techniques and confirmed to be wound induced by comparison with a cDNA library prepared from wound-induced mRNA. The gene promoter will be fused with the CAT gene using published techniques and incorporated into a transformation vector to transform alfalfa and tomato plants. The wound-induced promoter region will be modified and analyzed, using the transformation system to monitor CAT expression. The promoter will be assessed as a tool to genetically engineer plants to improve natural defense responses.

Progress: 87/01 to 87/12. Isolation and characterization of a wound-inducible proteinase inhibitor gene from alfalfa leaves is in progress. This gene is induced by insect attacts and is part of the defensive response of alfalfa against pests. Messenger RNAs containing wound-inducible mRNAs coding for a Bowman-Birk-type trypsin inhibitor (ATI) were isolated from wounded alfalfa leaves. cDNAs codign for the ATI mRNA were prepared from the mRNAs and identified by hybridization with a 32PO(4)-labeled 17 pb oligonucleotide probe prepared fom the known amino acid sequence of the N-terminus of the protein. The complete nucleotide sequence of one cDNA was determined. The cDNA contained the complete open reading frame of the inhibitor. This cDNA was employed to screen a genomic library of alfalfa to identify a genomic clone. The cDNA was also employed in hybridization experiments to assay levels of mRNA in alfalfa leaves in response to wounding and to determine tissue specificity of the expression of the ATI gene in alfalfa plants. The ATI mRNA is strongly wound inducible in leaves but only weakly in stems. The inhibitor is strongly expressed constitutively in the roots and fruit but only weakly expressed in shoots. In the field, the gene is expressed in low levels in leaves. When wounded, the leaves accumulate large amounts of ATI mRNA within 24 hr. The characterization of the wound--inducible ATI gene is in progress.

Publications: 87/01 to 87/12

BROWN, W.E. and RYAN, C.A. 1984. Isolation and characterization of a wound-induced trypsin inhibitor from alfalfa leaves. Biochem. 23:3418-3422.

BROWN, W.E., TAKIO, K., TITANI, K. and RYAN, C.A. 1985. Wound-induced trypsin inhibitor in alfalfa leaves: identify as a member of the Bowman-Birk inhibitor family. Biochem. 24:2105-2108.

BROWN, W.E., GRAHAM, J.S., LEE, J. and RYAN, C.A. Regulation of synthesis of proteinase inhibitors in food plants. In Freidman, M. (ed) "Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods." Plenum Press (in press).

20.016* CRISO099212
MOLECULAR GENETIC CHANGES ASSOCIATED WITH
SELECTION IN AGRONOMIC CROPS

OSBORN T C; Agronomy; University of Wisconsin, Madison, **WISCONSIN** 53706.

Proj. No.: WISO3068 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 86 to 30 SEP 90

Objectives: The overall objective is to obtain molecular evidence for the generation of new genetic variation in agronomic crops. The specific objectives are to 1) characterize changes in the copy number of repeated DNA sequences in maize populations under selection and in alfalfa lines derived from tissue culture, 2) determine if changes in repeated DNA are associate with phenotypic variation and 3) study the inheritance of variation in repeated DNA copy number.

Approach: Genomic DNA libraries of a selected maize population and of an alfalfa line used for tissue culture regeneration will be screened by differential hybridization using the DNAs isolated for cloning and DNAs from a divergently selected population (maize) or tissue culture derived variants (alfalfa). Differentially hybridizing DNA clones will be used as probes on slot blots and Southern blots containing DNAs of the starting inbred lines and the intermediate and advance selections (maize) or the parent and tissue culture derived lines (alfalfa). Changes in the copy number of repeated DNA sequences that are correlated with phenotypic changes will be analyzed in segregating progenies of maize and alfalfa. Thus the inheritance of repeated DNA copy number and its relationship to new phenotypic variation will be determined.

Progress: 88/01 to 88/12. This is a summary of progress in the molecular characterization of genetic variation in three different plant systems. The first system deals with new genetic variation that arises in alfalfa tissue culture. We have identified several repeated DNA sequences that have changed copy number in somaclonal variants. These changes occur readily in culture and they maybe associated with phenotypic variation arising in the culture process. These sequences have been cloned and the clones provide tools for further investigations on the molecular origins of somaclonal variation. The second system is the organization and expression of genes encoding arcelin seed protein in common bean. We have backcrossed arcelin genes from wild into cultivated beans and expression of arcelin in these lines confers high levels of bruchid beetle resistance. We have partially characterized alleltic variants of arcelin to determine their relationship to each other and to the related seed protein, phytohemagglutinin (PHA). Arcelin genes appear to have arisen by duplication of PHA genes to create a complex gene family. The third system involves genome characterization in Brassica species. We have used molecular markers (RFLPs) to study evolutionary relationships and to construct genetic linkage maps. These markers also are being used to identify and characterize genes controlling morphological variation.

Publications: 88/01 to 88/12
FIGDORE, S.S., KENNARD, W., SONG, K.M.,
SLOCUM, M.K. and OSBORN, T.C. (1988)
Assessment of the degrees of restriction
fragment length polymorphism in Brassica.
Theor. Appl. Genet. 75:833-840.

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Theor. Appl. Genet. 75:833-840.

HARTWECK, L.M. and OSBORN, T.C. (1988) Origin of genes encoding arcelin, an insecticidal seed protein of Phaseolus vulgaris. Genome 30. suppl. 1:136.

30, suppl. 1:136.
KIDWELL, K.K. and OSBORN, T.C. (1988) Changes in repeated DNA sequences in alfalfa somaclones. Agron. Abstr. p. 169.

OSBORN, T.C., ALEXANDER, D.C., SUN, S.S.M., CARDONA, C. and BLISS, F.A. (1988)
Insecticidal activity and lectin homology of arcelin seed protein. Science 240:207-210.

OSBORN, T.C., BUROW, M. and BLISS, F.A. (1988) Purification and characterization of arcelin seed protein from common bean. Plant Physiol. 86:399-405.

SLONG, K.M., OSBORN, T.C. and WILLIAMS, P.H. (1988) Brassica taxonomy based on nuclear restrication fragment length polymorphisms (RFLPs) 1. Genome evolution of diploid and amphidiploid species. Theor. Appl. Genet. 75:784-794.

SONG, K.M., OSBORN, T.C. and WILLIAMS, P.H. (1988) Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs) 2. Preliminary analysis of subspecies within B. rapa (syn. Campestris) and B. oleracea. Theor. Appl.

CM 21 COTTON

21.001 CRISO057686 PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON

ENDRIZZI V; Plant Science; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-137105-R-25-027

Project Type: HATCH

Agency ID: CSRS Period: 09 JAN 86 to 30 SEP 91

Objectives: To investigate the cytogenetics of cotton through the study of chromosome behavior, structure, and to determine the contributions of individual chromosome sets to plant characteristics and development; to integrate data from cytology, genetics, comparative morphology, and other disciplines into an improved taxonomic understanding of Gossypium and its relatives.

Approach: Monosomes, telosomes, and isosomes will be isolated, identified, and established in a common genetic background. Translocations consisting of known chromosomes will be crossed to the deficient lines to identify the aberrant chromosome. The chromosome aberrations will be used to locate genetic characters, linkage groups and centromeres on specific chromosomes.

Progress: 70/07 to 88/09. Accomplishments of the project for the period of July 1970 to September 1988. Eight of 15 monosomic chromosomes isolated and identified. Thirty-one telocentric chromosomes: for one or both arms of nineteen chromosomes isolated and identified. Seven linkage groups of 2 or more genes were established. Individual genes and linkage groups assigned to eleven chromosomes. One or more genes assigned to specific arms of nine chromosomes. Six new mutant genes and four chromosome translocations were identified and added to the regional collection. TERMINATED 30 SEP 88.

Publications: 70/07 to 88/09
No publications reported this period.

21.002 CRISO034275 DEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON

MENZEL M Y; Biological Sciences; Florida State University, Tallahassee, **FLORIDA** 32306.

Proj. No.: 8400035 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 84 to 30 SEP 86

Objectives: PROJ 8400035. To develop a system of deficiency and duplication chromosome markers for cotton, and to identify marker genes for as many of these chromosomes as possible, with special emphasis on chromosomes 8,11,13,14,19,21,23, and 24, for which no monosomes, telosomes, or marker genes have been identified

Approach: Previously, the breakpoints in 58 reciprocal chromosome translocation lines were mapped to their chromosome arm and distance from the centromere, and the complementary adjacent-1 duplication-deficiencies were generated and characterized as to frequency, morphological stigmata if any, and cytological

characteristics. In this phase of the work, we will reconcile "right" and "left" arms defined by the translocation breakpoints with "long" and "short" arms defined by telosomes; use deficiencies to map previously unmapped marker genes; attempt to position known linkage groups relative to appropriate breakpoints. We will also try, using in vitro culture methods, to find a way to enhance recovery of some of the rarer deficiencies.

Progress: 85/09 to 86/09. The 58 reciprocal translocations that constitute the primary reference set of chromosome markers are being exploited as a source of chromosome deficiencies. A map of the 116 breakpoints (bp's) based on arm location and interstitial chiasma frequencies was constructed (Menzel, Richmond and Dougherty 1985. J. Hered. 76:406-414). Duplication-deficiencies (dp-df's), mostly of the adjacent-1 type, were generated from 56 of the translocations and characterized morphologically and cytologically . A total of 107 types were found, of which 101 could be tentatively assigned to their specific cytotypes. Frequencies of dp-df recovery differs widely among translocations and, surprisingly, were not significantly correlated with map lengths of the missing or duplicated segments, but appeared to be idiosyncratic, perhaps governed by dosage interactions between the specific duplicate and deficient segments, . Present research focuses on (1) reconciling arm locations of bp's with Long and Short arms defined by telosomes (completed for 38 bp's and for chromosomes 5, 9, 14, 15, 16, 17, 18 and for chromosome 20 except for bp-2791), and (2) placing marker genes and linkage groups relative to bp's (completed for R(1) on 16 and nearly completed for L(2)0 and 15). 166 tests of the unassigned genes Y(1), v(3), v(4), v(9), g(1), and vf against previously untested chromosomes revealed no new associations, but tests are continuing.

Publications: 85/09 to 86/09

MENZEL, M.Y., HASENKAMPF, C.A., DOUGHERTY, B.J., RICHMOND, K.L. and CAMPBELL, L.B. 1986. Characteristics of duplications and deficiencies from chromosome translocations in Gossypium hirsutum. J. Hered. 77:189-201.

MENZEL, M.Y. and RICHMOND, K.L. 1986. A revised map of chromosome 15 in Gossypium hirsutum. Can. J. Genet. Cytol. 28:272-277.

RHYNE, C.L., RHYNE, D.M. and MENZEL, M.Y. 1986. Duplicate pollen-sterile locus msg and linkage group V of Gossypium hirsutum. J. Hered. 77:332-336.

CONTOLINI, C.S. and MENZEL, M.Y. 1987. Early development of duplication-deficiency ovules in Gossypium hirsutum. Crop. Sci. In Press.

MENZEL, M.Y. and DOUGHERTY, B.J. Transmission of duplication-deficiencies from cotton translocations is unrelated to map lengths of the unbalanced segments. Accepted (Genetics).

21.003 CRISO034214 GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY

GALAU G A; Botany; University of Georgia, Athens, **GEORGIA** 30602.

Proj. No.: GEO-8400684 Project Type: CRGO Agency ID: CRGO Period: 15 JUL 84 to 31 JUL 86

Objectives: PROJ 8400684. We wish to study the genetic and molecular basis of an alloplasmic-genetic male sterility which resulted when the Gossypium hirsutum nuclear genome was transferred into G. harknessii cytoplasm. Restoration of fertility is dependent on maintenance of a restorer gene, Rf, presumed to derive from G. harknessii.

Approach: We wish to create, via semigamy-mediated transfers, reciprocal combinations of cytoplasm and nuclear genomes of these species. Using these constructions, isogenetic lines under construction, and current diverse lines, we will examine leaves and anthers for alterations in gene structure or expression which correlate with phenotype. Analysis will be by restriction enzymes, in vivo, in organello, and in vitro protein synthesis.

21.004 CRISO138365 POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS

MALLET J L B; Entomology; Mississippi State University, Mississippi State, **MISSISSIPPI** 39762.

Proj. No.: MIS-2122 Project Type: HATCH
- PENDING

Agency ID: CSRS Period: O1 JUL 89 to 30 JUN 94

Objectives: Estimation of migration and gene flow parameters in the tobacco budworm Heliothis virescens, mapping insecticide resistance in H. virescens in the state of Mississippi; development of approaches to resistance management.

Approach: Gene flow will be estimated by direct estimates of dispersal in mark-release experiments and by determining spatial variation in gene frequencies, both of allozymes, and of genes involved in insecticide resistance. H. viresens will be collected statewide and subjected to discriminating doses of pyrethroids; modeling and field data will be integrated to help provide viable programs of resistance management.

21.005* CRISO049430 GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS

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STEINER W M M; Parasite-predator Bio & Ecol Bio Control of Insects Lab; Agricultural Research Service, Columbia, **MISSOURI** 65211. Proj. No.: 3622-24000-006-00D

Project Type: INHOUSE Agency ID: ARS Period: 01 OCT 84 to 30 SEP 89

Objectives: Genetically map and characterize chromosomal, morphological, DNA, and allozyme variants of selected entomophagous insects and determine their physiological, developmental and behavioral significance and their potential for utilization in bioengineering programs to enhance entomophageefficiency.

Approach: Populations of selected entomophages will be assayed using classical gene- tic techniques and restriction enzyme (DNA) technology to establish gene- tically typed isolines. These will be used to establish linkage maps and study genome structure in the context of predator-parasite/prey models. About 80 % of effort will be devoted to the model offered by Heliothis zea and its entomophages with 20% of effort reserved to develop genetic know- ledge of other models. Selected variants will also be biochemically char- acterized and their distributions in natural populations determined. Lab- oratory tests of physiological, developmental and behavior response under stresses posed by pesticides, temperature and desiccation will reveal whichphenotypes can enhance natural population fitness characteristics. Methods of transferring desirable traits will be developed.

Progress: 88/01 to 88/12. Selection for insecticide resistance in females of the parasitoid Microplitis croceipes was initiated to determine how amenable this parasite of Heliothis spp. is for development of insecticide resistant varieties. After 5 generations of inbreeding, two of five lines demonstrated an increase in LD values, going from 0.82 ug fenvalerate (in 0.5 ul acetone topical application) to a 16-fold increase at 13.12 ug with an accompanying increase in number of males being produced and a decline in line fertility. Selection ceased at the 6th round when the lines were lost due to the increased selection pressure. The observed selection response is a typical one seen for braconid wasps, and suggests this is the upper limit to which the M. croceipes genome may respond to insecticide selection. In other studies, three species of nabids were found to segregate for allozyme variation at an esterase locus (EST-1!) and an adenylate kinase locus (ADK-3). These were not in genetic equilibrium and reduction in the numbers of heterozygotes in nature suggests strong population subdivision. These loci may serve a diagnostic function to differentiate closely related nabid species.

Publications: 88/01 to 88/12
GRASELA, J.J., STEINER, W.W.M. and MARSTON,
 N.L. 1988. Genetic differences at two
 allozyme loci in midwest populations of

three species of Nabidae. Comp. Biochem. Physiol. 90B:427-431.

STEINER, W.W.M. 1988. Electrophoretic techniques for the genetic study of aphids In, A.K. Minks and P. Harrewijn (Eds.) "Aphids, Their Biology, Natural Enemies and Control", Vol. B, Elsevier Sci. Publ., Amsterdam, pp. 135-143.

21.006* CRISO133477 SOMATIC CELL GENETICS IN VEGETABLE CROPS

O'CONNELL M A; Agronomy & Horticulture; New Mexico State University, Las Cruces, **NEW MEXICO** 88003.

Proj. No.: NM-1-5-27411 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 87 to 30 SEP 92

Objectives: 1) Construct and characterize cybrids, asymmetric and complete somatic hybrids of tomato. 2) Evaluate feasibility of objective 1 with other vegetable crops, onion and pepper. 3) Characterize mitochondrial genome of tomato. 4) Characterize heat shock response in heat sensitive and resistant lines of cotton. 5) Generate and utilize RFLP maps in vegetable crops.

Approach: 1) Protoplasts and cytoplasts will be prepared from appropriate explants of cultivars of tomato and from wild relatives of tomato. The protoplasts or cytoplasts will be fused, and the products regenerated into plants. The regenerants will be characterized using nuclear genome and organellar genome RFLPs, isozyme activities, and morphology and fertility. 2) Transfer of protoplast technology to the recalcitrant crops, onion and pepper will be tried. 3) Libraries containing tomato mitochondrial DNA have been prepared. The recombinant clones will be mapped with restriction enzymes, and a physical map of the genome constructed. Genes will be located on the map using heterologous probes and Northern analysis. 4) The heat shock proteins synthesized by heat tolerant and sensitive lines will be determined by in vivo protein synthesis experiments and analysis of the radioactive prOteins on 2-dimensional gels.

Progress: 87/09 to 88/12. The chloroplast DNA of tomato cultivar Cal Ace was used to generate a library of the Sal 1 fragments of the genome. These cloned fragments are now being used to map the chloroplast genome of pepper, Capsicum annuum. In addition, the chloroplast DNA from several species of Capsicum have been purified and analyzed on agarose gels. These results will allow us to generate a taxonomic relationship between the species based on RFLPs in the chloroplast genome. Comparisons were made between the expression of head shock proteins (HSPs) in genetically characterized heat tolerant and heat sensitive lines of cotton. These comparisons were based on electrophoretic analysis of in vivo labelled proteins. Several HSPs were identified on two dimensional gels which were expressed uniquely in either the tolerant (26 kDa) or sensitive cotton line (24 kDa and 18 kDa). However, the HSP pattern displayed in a heat tolerant BC-3 individual

was that of the heat sensitive parent. The expression of HSPs in two species of tomato, Lycopersicon esculentum and L. pennellii, and the interspecific hybrid were characterized. Unlike the results obtained in the cotton studies, major differences in the sizes of the classes of HSPs expressed by these sexually compatible species were observed.

Publications: 87/09 to 88/12
No publications reported this period.

21.007* CRISO034080 THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN AND COTTON

TU C P D; GROVE G; LI N; Biochemistry Microbiology Molecular & Cell Biology; Pennsylvania State University, University Park, PENNSYLVANIA 16802.

Proj. No.: 8300456 Project Type: CRG0 Agency ID: CRG0 Period: 01 SEP 83 to 31 AUG 86

Objectives: Project Number 8300456. We inend to study the molecular genetics of the glutathione S-transferase (GST) genes from corn and cotton. We will isolate the GST isozymes from corn, prepare antisera against corn GSTs, construct cDNA plasmids for corn GST mRNA sequences and isolate genomic sequences from constructed gene libraries of corn and cotton. Our long-term goal is to establish the relationship between GST isozymes expression and selected herbicide resistance (e.g. atrazine resistance) in corn. The GST gene sequences can be manipulated in vitro for their expression in heterospecific environments.

Approach: The GST isozymes will be purified by a procedure involving S-hexylglutathione linked sepharose 6B affinity column chromatography. The GST cDNA plasmids will be characterized by hybrid-selected in vitro translation and DNA sequence analysis. Genomic sequences will be isolated from gene libraries with specific cDNA probes. The GST isozyme(s) involved with atrazine resistance will be identified and characterized from an atrazine sensitive corn line.

Progress: 86/09 to 87/11. We have isolated from a constructed lambda gtll expression library two classes of cDNA clones encoding the entire sequence of the maize GSH S-transferases GST I and GST III. Expression of a full-length GST I cDNA in E. coli resulted in the synthesis of enzymatically active maize GST I that is immunologicaly indistinguishable from the native GST I. Another GST I cDNA with a truncated N-terminal sequence is also active in heterospecific expression. Our GST III cDNA sequence differs from the version reported by Moore et. al (Moore, R. E., Davies, M. S., O'Connell, K. M., Harding, E. I., Wiegand, R. C., and Tiemeier, D. C. (1986) Nucleic Acids Res. 14:7227~7235) in eight reading frame shifts which result in partial amino acid sequence conservation with the rat GSH S-transferase sequences. The GST I and GST III sequences share cycle sine 45% amino acid sequence homology. Both the GST I and the GST III mRNAs contain different repeating motifs in front of the initiation codon ATG. Multiple poly(A) addition sites have been identified for these two classes of maize GSH S-transferases messages. Genomic Southern blotting results suggest that both GST I and GST III are present in single or low copies in the maize (GT112 RfRf) genome. We have achieved the original objectives of the proposal: to purify GST isozymes from corn, to prepare antisera against these purified maize GST isozymes, to construct cDNA libraries (lambda gt11 vector and plasmid vectors), to isolate and characterize GST cDNAs, to express corn cDNAs in E.

Publications: 86/09 to 87/11

- TIMMERMAN, K.P. and TU, C.-P.D. 1987. Genetic evidence of xenobiotics metabolism by GSH S-transferases from corn in "Glutathione S-Transferases and Carcinogenesis" pp 47-49, Edited by T.J. Mantle, C.B. Pickett and J.D. Hayes.
- TIMMERMAN, K.P. 1987. Purification and characterization of corn glutathione S-Transferases, Ph.D. Thesis. The Pennsylvania State University, University Park, PA. 93 p.
- GROVE, G., ZARLENGO, R.P., TIMMERMAN, K.P., LI, N., TAM, M.F. and TU, C.-P.D. 1988. Characterization and heterospecific expression of cDNA clones of genes in the maize GSH S-transferase multigene family. Nucleic Acids Res. 16:(in press).

21.008 CRISO041300 GENETICS, CYTOGENITCS OF COTTON GERMPLASM

KOHEL R J; Agricultural Research Service; Agricultural Research Service, College Station, TEXAS 77843.

Proj. No.: 6202-20061-003-00D

Project Type: INHOUSE Agency ID: ARS Period: 21 AUG 74 to 30 SEP 85

Objectives: Determine the genetics of qualitative characters in cotton. Establish linkage relations among genes to identify genetically the chromosomes of cotton. Maintain collection of mutant germplasm.

Approach: Assemble mutant stocks and lines with special characters derived from spontaneous mutations, induced mutations, races, and species. Conduct investigations of mutagens on cotton with primary emphasis on chemical mutagens. New or unanalyzed mutants will be analyzed genetically for inheritance and linkage relations and tested for monosome association. These mutants will be converted to isolines, entered into the mutant $\operatorname{\mathsf{germplasm}}$ collection, and made available to other scientists. The mutant germplasm collection will be increased for storage in the National Seed Storage Facility. Marker genes will be combined into multiple marker lines and linkage group tester lines. Cooperate with cytogeneticist to coordinate genetic and cytological identification of chromosomes. i i d.

Progress: 74/08 to 85/10. Three-hundred eighteen linkage combinations were tested that revealed four new linkage groups, added ten

mutants to existing linkage groups, and found no associations in the remaining combinations./ Eighty mutant-monosome combinations were tested that identified two new associations, placed four loci on existing linkage groups, and revealed independent associations in the remainder./ Nineteen potential mutants were tested and seven new mutants were identified./ A chemical mutagen was used to induced new mutations, but no new mutants were recovered./ The development of isolines of all new mutants were included in the isoline program. / Linkage group stocks were developed for all new linkage groups./ Tests were run to establish gene order in linkage groups./ Cotton somaclones contained a high frequency of cytogenetic abnormalities.

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Publications: 74/08 to 85/10 NO PUBLICATIONS REPORTED THIS PERIOD.

21.009* CRISO133059 FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR IDENTIFICATION AND CONTROL OF INSECTS

JOHNSON J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6916 Project Type: HATCH Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

Objectives: 1) Develop flow cytometry and in particular total nuclear DNA content determination as a cytotaxonomic tool, for the recognition of host races, biotypes, and crytic species of insects. 2) Catalogue the nuclear DNA content of insects of economic interest. 3) Develop multiparameter DNA flow cytometry using dyes which are specific for total DNA, adenine and thymine rich repetitive DNA, and guanine and cytosine rich repetitive DNA. 4) Demonstrate the developmental pattern and mode of inheritance of DNA content variation for both euchromatic (unique) and heterochromatic (repetitive) insect DNA sequences. 5) Develop methodology to sort insect nuclei and chromosomes as an aid to gene cloning in insects.

Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

Progress: 88/01 to 88/12. We have now had one year of work with this unique new methodology for the study of heritable variation in insects. We have measured the DNA content of over 1500 individual fire ants including 3 native and two imported species. In addition, we have measured the DNA content of over 200 honey bees, nearly 300 boll weevils, plus smaller number of individuals from 10 other species. The results have been unexpected and exciting. In particular we find: DNA content is a diagnostic tool that aids in identification of species, populations and types. To date, very significant and predictable DNA content differences have been found between 5 fire ants species, between 4 species of boll weevil, and between the Africanized form of the honey bee, the European

honey bee and the F-1 hybrid between the two forms. DNA content variation exists within and among some populations. The most extreme variation we have observed occurs in a fire ant population in Walton Co., Georgia, were 129 of 515 males, females, and workers show 3N or triploid DNA amounts. The remainder are diploid or haploid as expected. The smallest significant variation occurs between populations of the boll weevil Anthonomus grandis where DNA content changes are associated with different host plants. DNA change occurs during development. Male fire ants are haploid during early development, but double their DNA before maturity. Boll weevils are largely 2N throughout their life, although in 1/4 to 3/4 of their cells may have 15 to 20 percent additional DNA.

Publications: 88/01 to 88/12

JOHNSTON, J.S., ELLISON, J.R. and VINSON, S.B. 1989. Flow cytometric determination of insect DNA as an aid to the description, identification, and control of the imported fire ant. Vinson SB McCOWN JL (eds.) Proc. Imported Fire Ant Sympo.

JOHNSTON, J.S. and ELLISON, J.R. 1989. DNA heterogeniety within and among fire-ants of the genus Solenopsis in southern United States: Evidence from flow cytometry. Submitted to Cytometry.

JOHNSTON, J.S. and ELLISON, J.R. 1988.
Triploidy in a polygynous population of the imported fire ant Solenopsis invicta in Walton Co., Georgia. Submitted to Hereditas.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. A method for determination of DNA content of nuclei of insects by flow cytometry. Submitted to Cytometry.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

21.010* CRISO095101 ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS

KENERLEY C M; STACK J P; THOMAS M D; Plant Pathology & Microbiology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6733 Project Type: HATCH Agency ID: CSRS Period: 24 JAN 89 to 31 DEC 93

Objectives: A) Improve the biocontrol attributes of isolates of Gliocladium roseum, G. virens, and G. catenulatum using DNA transformation, protoplast fusion, and other selection procedures. B) Use transformed strains of Gliocladium species that have dominant, stable molecular markers to examine the mechanisms of antagonism. C) Assess the role soil edaphic factors have on propagule formation, survival and dispersal of the target pathogens, and enhanced or engineered antagonists. D) Identify DNA probes that can be used to distinguish the Gliocladium species and strains by RFLP analysis and to physically map selected sites on chromosomes.

Approach: A DNA transformation system developed for Gliocladium spp. will be used to introduce genes (fungicide-resistant, antibiotic production, fungal cell wall degradation) into selected strains of Gliocladium for enhancing biocontrol attributes. Genes will be selected by shotgun cloning by complementation or from other existing fungal systems. Protoplast fusion will be used in cases where genes or metabolic pathways have not been identified (chlamydospore production, sporulation ability, growth potential in soil). Probes for RFLP analysis will be developed from random cloning of genomic digests of Gliocladium spp. as well as using characterized genes cloned from other fungi. The formation of survival propagules and dispersal of fungi mycelium of engineered biocontrol antagonists as well as target hosts will be assessed in soil temperature tanks with a micro-video system.

Progress: 88/01 to 88/12. Protoplast of the biocontrol fungus Gliocladium virens have been transformed with the 6.7 kb plasmid pH1S containing a bacterial hygromycin resistance gene, hygB. Transformants were selected on medium containing 250 mu g/ml hygromycin. Total DAN was isolated from several transformants, digested with restriction endonucleases. blotted to nylon membranes, and hybridized to either purified hygb or pBR322 (vector)DAN. In most cases the hygB DNA was integrated into high molecular weight DAN and the vector DNA was not present. Also, an episomal, supercoiled, circular plasmid which autonomously replicates has been observed. The plasmid, pJJ31, arose from a spontaneous rearrangement of hygB that had integrated into the genome. It contains new cloning sites for Sal I and Bam HI and can act as a shuttle vector between E. coli and G. virens. Analysis of fusion products of intrastrain crosses of G. roseum revealed that up to six successive hyphal and/or conidial transfers were required to obtain colonies of a homogenous phenotype. All of the tested fusion products were of parental phenotype except for 15 stable His (Asn) and on medium containing benlate and on a medium containing benlate and acriflavin. Of the fusion products tested for their ability to colonize sclerotia of Phymatotrichum omnivorum, 3 performed as well as the control wild-type strain 1620. Several biocontrol agents knows to be antagonistic to Pythium sp. were evaluated for control of damping-off in Amaranthus cruentus by Pythium myriotylum.

Publications: 88/01 to 88/12

SEH, M.L. and KENERLEY, C.M. 1988. Protoplast formation and regeneration of three Gliocladium species. J. Microbiol, Methods 8:121-130.

SEALY, R.L., KENERLEY, C.M. and MCWILLIAMS, E.L. 1988. Evaluation of Amaranthus accessions for resistance to damping-off by Pythium myriotylum. Plant Dis. 72:958-989.

MARTYN, W.R., MORGAN, P.W., STERLING, W.L. and KENERLEY, C.M. 1988. Cotton fleahopper and associated microorganisms as components in the production of stress ethylene by cotton. Plant Physiol. 87:280-285.

KOCH, D.O., JEGER, M.J., GERIK, T.J. and KENERLEY, C.M. 1987. Spatial dynamics of Phymatotrichum root rot of row crops in the Blackland region of north central Texas. Phytopathology 77:1657-1662.

JEGER, M.J., KENERLEY, C.M., GERIK, T.J. and KOCH, D.O. 1987. Spatial dynamics of Phymatotrichum root rot in row crops in the Blackland region of north central Texas. Phytopathology 77:1647-1656.

21.011 CRISO093070 PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM

LYDA S D; Plant Pathology & Microbiology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6683 Project Type: HATCH Agency ID: CSRS Period: O2 MAY 84 to O1 MAY 89

Objectives: Study physiology of parasitism of Phymatotrichum omnivorum; Investigate physiological effects of specific chemicals on P. omnivorum, and Study ecology of P. omnivorum as influenced by environmental factors.

Approach: Basic studies on physiology of parasitism will be conducted in axenic and monoaxenic systems to elucidate toxin transfer during pathogenesis. Selected chemicals, especially triazole derivatives, will be evaluated for their efficacy in inhibiting vital processes in the life cycle of P. omnivorum. Slow release formulations prepared in starch xanthide and starch borates will be compared with commercial, granular and emulsifiable concentrate formulations. Translocation and persistence tests will be conducted with the radioactive preparations of the chemicals. These chemicals will be studied as possible control agents under greenhouse and field conditions. Studies on environmental factors affecting sclerotial production will be conducted in quartz model systems.

Progress: 88/01 to 88/12. Total DNA from 21 isolates of Phymatotrichum omnivorum, from alfalfa, apple, cotton, grape, maple, peach, and okra, were analyzed for restriction fragment-length polymorphisms following digestion with selected endonucleases. Band patterns on agarose gels were used to separate genetically distinct isolates. Quantity of sclerotia, size of sclerotia, and pathogenicity (aggressiveness) were discriminating criteria studies for all isolates. Data is being analyzed by similarity matrices and phenograms as a measure of relatedness. A toxin, isolated from germinating sclerotia and culture filtrates of P. omnivorum, has been purified and partially characterized. Preliminary data indicate the toxin is a peptidoglycan. It is lethal to cotton cell suspensions at concentrations comparable to fusaric acid. Cell viability was measured spectrophotometrically with a tetrazolium chloride procedure. Three triazole fungicides (flusilazole, penconazole and propiconazole) were evaluated at 0.1. 0.25 and 0.5 lb a.i./ac for efficacy in controlling Phymatotrichum root rot (PRR) at three location in Texas (Corsicana, Pearsall, and Prosper). The chemicals were applied in the covering soil at 20 gals solution/ac. A significant increase in seedling emergence was noted at Pearsall

with the 0.1-lb rate of all three fungicides. A reduced stand and stunting were observed at the 0.5-lb rate at Pearsall.

Publications: 88/01 to 88/12

MABELLOS, J.D. and LYDA, S.D. 1988. Selection of Phymatotrichum root rot resistance through tissue culture techniques. Cotton Dis. Council 48:37-39.

PANHWAR, G.A., LYDA, S.D. and RIGGS, J.L. 1988. Efficacy of contemporary fungicides in controlling seedling diseases of cotton. Cotton Dis. Council 48:20-22.

RIGGS, J.L. and LYDA, S.D. 1988. Laboratory and field tests with triazole fungicides to control Phymatotrichum root rot of cotton. Cotton Dis. Council 48:45-48.

WU, C.H. and LYDA, S.D. 1988. Applications of restriction fragment length polymorphisms in studying genetic variation of Phymatotrichum omnivorum. Cotton Dis. Council 48:39-41.

21.012 CRISO136595 DEVELOPMENT OF A MOLECULAR CYTOGENETIC MAP OF COTTON BY IN SITU HYBRIDIZATION

PRICE H J; MCKNIGHT T D; STELLY D M; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6963 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 88 to 31 AUG 91

Objectives: PROJ. 8800583. The goal of this research is to begin development of a molecular cytogenetic map of the genue Gossypium. The specific main objectives of this work will be to develop the DNA clones and molecular cytogenetic technology for studying intergenomic differences and intergenomic introgression.

Approach: Repetitive sequences from G. sturtianum (2n=2x=26; C, genome) will be cloned, screened for repetiveness, dispersiveness and distinctiveness from G. hirsutum repetitive sequences, and then hybridized singly or as sets of clones to determine which provide good "coverage" of the C. genome. Existing in-house techniques for in situ hybridization (ISH) will be tailored to the project's needs. Identified clones and ISH techniques will be applied to backcross-introgression products to determine effectiveness of ISH techniques and introgression.

Progress: 88/09 to 88/12. Procedures were established for in situ hybridization (ISH) of biotinylated rDNA sequences to cotton meiotic chromatin. Procedures for coating slides were subsequently improved. Nucleolar organizing regions (NORs) were detected on three chromosomes of the haploid complement. Utility of ISH in combination with cytogenetic stocks was demonstrated for molecular cytogenetic mapping; one NOR was mapped to chromosome are 9L, using translocation heterozygotes and an 18S rDNA probe. About half of the remaining complement was shown not to contain either remaining NOR. Repetivie sequences from G. sturtianum, a C-genome diploid of Australia

were cloned and several have been tentatively identified as unique relative to A and D genomes of G. hirsutum. Tests to verify preliminary results are underway.

Publications: 88/09 to 88/12

BERGEY, D.R., PRICE, H.J., STELLY, D.M. and MCKNIGHT, T.J. 1988. In situ hybridization of a cloned ribosomal RNA gene to meiotic chromosomes of cotton. AIBS BSA p. 98.

BERGEY, D.R., STELLY, D.M., PRICE, H.J. and MCKNIGHT, T.D. 1988. In situ hybridization of biotinylated DNA probes to cotton meiotic chromosomes. Agron. Abstr. p. 165.

BERGEY, D.R., STELLY, D.M., PRICE, H.J. and MCKNIGHT, T.D. (submitted 5/88) In situ hybridization of biotinylated DNA probes to plant meiotic chromosomes. Stain Technol. (accepted).

21.013 CRISO034284 GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON

STELLY D M; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6780 Project Type: CRGO Agency ID: CRGO Period: 15 SEP 84 to 31 AUG 86

Objectives: PROJ 8400547. Map the semigamy locus to a chromosome or linkage group in cotton. Re-determine the inheritance of semigamy in cotton; develop new genetic and cytogenetic stocks of cotton, involving or using semigamy; begin a detailed genetic analysic of the time and mode of gene action at the semigamy locus.

Approach: Traditional genetic linkage and monosome analyses, modified as needed, will be used to map the semigamy locus. Additional cytogenetic stocks also will be used, according to availability. Patterns of segregation for the semigamy trait will be used to determine inheritance. Hybridization, recombination and progeny testing will be used to develop new stocks; derived quasi-haploid types will be colchinine doubled. The time and mode of semigamy allele action will be determined by analysing 3 x 3 factorial mating of SeSe, Sese, and sese parent sets, and behavior of heterozygous gametes.

Progress: 84/09 to 86/12. Experimental progress towards the genetic and biological characterization of the semigamy mutant of cotton is as follows: To determine the time and mode of gene action of the senigany mutant, 15,000 F(1) seed were produced from the factorial mating scheme; respective seedling evaluations are ongoing. If semigamy is determined to be gametophytically expressed, tests for the mode of gene action will require production of heterozygous gametes. Numerous putative octaploids produced by colchicine treatment were male-sterile, indicating duplication-deficiencies must be used to produce the requesite heterozygous gametes. To locate the locus, semigamous heterozygotes have been identified for the following chromatin-deficiency types: 1) monosomics for chromosomes 1, 2, 4, 7, 10, 12, 18, 22, 25 and

M60, 2) monotelodisomics for chromosome arms 15, 3L, 4S, 4L, 6S, 6L, 10S, 12L, 15L, 18L, 20S, 20L, 22S and 26S; and 3) several duplication-deficient types. Additional chromatin-deficient types selected phenotypically remain to be identified cytologically. Testcross progeny and their self-seed have been obtained for most of the chromosome-deficient types for seedling tests needed to complete this experiment. Tests to determine usefulness of the semigamy mutant to derive new aneuploid types in cotton also are in progress. Several off-type haploid progeny, possibly nulli-types, have been found to date.

Publications: 84/09 to 86/12
GWYN, J.J. and STELLY, D.M. 1986. The use of
 joint-scaling tests to determine the time
 and mode of gene action of reproductive
 mutants. Agron. Abstr. p. 65.

21.014 CRISO099648 GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON

STELLY D M; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX-8600412 Project Type: CRGD Agency ID: CRGO Period: 15 SEP 86 to 30 SEP 90

Objectives: PROJ 8600412. Map the semigamy locus to a chromosomic and linkage group. Determine time and mode of semigamy gene action. Determine if "nulli-types" of cytogenetic stocks can be derived via the semigamy mutant. Determine if cytoplasm or environment affects semigamy expression. Develop methods for mass extraction of double haploids of cotton.

Approach: Standard cytogenetic and genetic techniques. Factorial 3x3 mating, followed by joint-scaling tests; formation of diallelic gemetes. Derive nulli-type haploid progeny from aneuploid semigamous parents, then colchicine-double. Cytoplasmic substitution; field and growth chamber experiments. Combine Se and Le(2)dav.

Progress: 86/09 to 87/12. To determine the time and mode of gene action of the Semigamy mutant, more than 20,000 F(1) seedlings from a factorial mating of genetically marked semigamous parents have been evaluated. Detailed examination and computer analyses of data are yet to be completed. Resulting haploid and chimeric progenies are being studied and chromosome-doubling is being attempted. For chromosome mapping of the semigamy gene, BC(1)F(1) plants from chromatin-deficient types are being selfed and S(1) plants are being seedling tested. F(2) and testcross seed have been obtained to determine allelism and chromosome locations of the v(1) and v(7)genes. Seedling tests are to be made this winter and spring. A number of BC(1)S(1) plants and less advanced marker lines were phenotypically selected for general single-plant vigor and fecundity, as possible le(1) le(1) Le(2) Le(2)dav Se Se plants needed for development of the proposed HEHP system of mass-extraction of doubled haploids. Large

numbers of cross-pollination were made between genetically marked semigamous females Se Se v(7) v(7) and Le(2)dav Le(2)dav males to substantiate further the biological tenets employed in the HEHP system. Seedling tests are planned for the winter/spring. A limited survey of 53 adapted US. cultivars indicated allele frequencies for le(1) and le(2) are zero or close to it.

Publications: 86/09 to 87/12

GWYN, J.J. and STELLY, D.M. 1987. A statistical method to determine the time and mode of gene action of apomictic reproductive mutants. Pro. Ann. Meeting Texas Genet. Soc. Vol. 14 (Abstr.).

ROONEY, W.L. 1987. A survey of cotton (Gossypium hirsutum) for alleles le(1) and le(2). Univ. Undergrad. Fellow Thesis. Texas A&M University. 81 pp.

21.015 CRISO130211 GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON

STELLY D M; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843. Proj. No.: TEXO6878 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 86 to 30 SEP 89

Objectives: PROJ 8600412. Map the semigamy locus to a chromosomic and linkage group. Determine time and mode of semigamy gene action. Determine if "nulli-types" of cytogenetic stocks can be derived via the semigamy mutant. Determine if cytoplasm or environment affects semigamy expression. Develop methods for mass extraction of doubled haploids of cotton.

Approach: Standard cytogenetic and genetic techniques. Factorial 3x3 mating, followed by joint-scaling tests; formation of diallelic gametes. Derive mulli-type haploid progeny from aneuploid semigamous parents, then colchicine-double. Cytoplasmic substitution; field and growth chamber experiments. Combine Se and Le(2).

Progress: 88/01 to 88/12. Two techniques that might allow derivation of nullisomics were evaluated. No nulli-maternal haploids were obtained from more than 5000 self-seed of semigamous aneuploids; 1/350 progeny from limited pollinations were Te20s, indicating a low level of male transmission of this deficiency. Cytoplasmic substitution for semigamous line '57-4' into G. hirsutum cytoplasm was achieved; paternal haploid recovery has been low/nill for other cytoplasms. Octaploids from Se se tetrapolids have remained completely sterile; translocations or other cytogenetic stocks will be needed to generate fertile diallelic gametophytes to determine the mode of gametophytic gene action. Additional Se se or Se--cytogenetic deficiencies were recovered and test crossed to 'S6' (se se); five progeny of each were selfed and are being tested. Most testcross S(1) seedling tests have been completed and data are being summarized. Seedling evaluations for 20,000 F(1) seed from

factorial mating were completed; data are being summarized; matrical regression will be used to define times and modes of Se gene action, and relative importances. The Le(2)DAV hybrid necrosis reaction was shown to involve intracellular events. Low-to-zero frequencies of le(1) and le(2) discerned in certain test crosses of G. hirsutum germplasm, supporting the potential applicability of the proposed HEHP system for mass-extraction of doubled haploids.

Publications: 88/01 to 88/12 STELLY, D.M., LEE, J.A. and ROONEY, W.L. 1988. Proposed schemes for mass-extraction of doubled haploids of cotton. Crop Sci. 28: 885-590. ROONEY, W.L. and STELLY, D.M. Allelic composition of cotton (Gossypium hirsutum L.) at the Le(2) loci. Crop Sci. (accepted). STELLY, D.M. and ROONEY, W.L. Delimitation of the Le(s)_d_a_v complementary lethality system to intracellular interaction. J. Hered. (accepted). GWYN, J.J. and STELLY, D.M. 1988. A method to assess pollen fertility in cotton. Beltwide Cotton Prod. Res. Conf. p. 95. STELLY, D.M. and ROONEY, W.L. 1988. Delimitation of the Le(2)_d_a_v complementary lethality system of Gossypium to intracellular interaction. Agron. Abstr. ROONEY, W.L. and STELLY, D.M. 1988. Composition of cotton (Gossypium hirsutum L.) at the Le(1) and Le(2) loci. Agron.

21.016 CRISO057660 PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON

Abstr.

STELLY D M; KOHEL R J; FRYXELL P A; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO2771 Project Type: HATCH Agency ID: CSRS Period: 09 JAN 86 to 30 SEP 91

Objectives: To acquire, maintain, increase, and distribute a diverse and usable cotton germplasm collection. To characterize and evaluate the germplasm collections. To adapt and develop novel methodology to provide additional ways to modify and utilize the germplasm and to improve basic understanding of genetic expression. To investigate the genetics and enhancement of the cotton germplasm.

Approach: Aquisition, maintenance, systematic, taxonomic, cytogenetic, genetic and agronomic evaluations of germplasm, and hybrids therefrom. Use of chromosome aberrations, deficiency aneuploids, and genetic markers to improve the genetic map and study cotton cytogenetic composition. Biotechniques; including in vitro culture techniques, molecular DNA analyses, and isozyme analyses. Qualitative and quantitative genetics, for seed, plant, boll, fiber, disease, pest, and other traits.

Progress: 88/01 to 88/12. 'TM-l' isoline development of identified monosomic and monotelodisomics and substitution lines was advanced. G. hirsutum tissue cultures were found to ahave hypoaneuploidy and bridge formation. A scheme to render tertiary monosomics more usable was verified. Linkage tests for 47 mutant combinations revealed a potential new linkage of g12 with Li2. Condensed tannin concentration was found to express significant general combining ability. Data from 10F(2) populations involving race stock T-277-2-6 indicate that individual plants with boll weevil resistance can be identified. The Regional Short Season Test included 16 entries & planted at 8 locations. Fourteen germplasm lines of compact growth type were registered. The long-term floristic study of the Malvacaea of Mexico was completed. An ecological and phenetic study of Ciefuegosia intermedia and its evaluation as a potential alternate host plant of the boll weevil was completed. Gossypol/total terpenoid rates were shown to be less than 10% in leaves and 75% in flowerbuds. Exogenous hormone applications at pollination were shown to be equal to or superior to ovule/embryo culture for interspecific crosses. An in vitro method was developed to collect germplasm. Germplasm (108 accession/3 species) and information on cotton production in NE Brazil were collected. 900 accessions were increased at Tecoman, Mexico. Seed of 1193 accessions were distributed to 36 individuals.

Publications: 88/01 to 88/12

- ALTAMARION, T., SMITH, C.W., LOVE, J., SCHUSTER, M.F., BELL, A.A. AND STIPANIVAC, R. 1988. Progress in developing high tannin Cotton for Heliothis resistance. In J.E. Brown (ed.). Proc. Beltwide Cotton Prod. Res. Conf. 1988:553.
- ALTMAN, D.W. 1988. Exogenous hormone applications at pollination for in vitro and in vivo production of cotton interspecific hybrids. Plant Cell Rep. 7:257-261.
- ALTMAN, D.W., FRYXELL, P.A., KOCH, S.D. AND HOWELL, C.R. 1988. Supplemental in vitro method for field collection of wild Gossypium germplasm. In Vitro Cell.
- Devel. Biol. 24)3, II):56.
- AYRES, N.M., ALTMAN, D.W. AND THOMAS, M.D. 1988. Analysis of the chloroplast DAN of cotton to establish evolutionary relationships. Beltwide Cotton Prod. Res. Conf. p. 98.
- BATES, S.L., WALKER, J.K. AND SMITH, C.W. 1988. Detecting boll weevil resistance in converted cotton race stocks by sampling single plants. In. J.E. Brown (ed.). Proc. Beltwide Cotton Prod. Res. Conf. 1988:552.
- FRYXELL, P.A. 1988. The Australian species of Pavonia. Nuytsia (published, but citation not yet available).
- FRYXELL, P.A. 1988. "Charles Wright on the Boundary, 1849-1852, or Plantae Wrightianai Revisited" by Elizabeth A. Shaw. Econ. Bot. 42:53. (book review).

23.001 CRISO099202 REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSION

GOLDBERG R B; Biology; University of California, Los Angeles, CALIFORNIA 90024.

Proj. No.: CALR-8601843 Project Type: CRGD Agency ID: CRGO Period: 01 JUL 86 to 30 JUN 89

Objectives: PROJ 8601843. The purpose of this research is to uncover the cis-control elements that regulate lectin gene expression and to identify trans-factos that interact with those elements.

Approach: DNA gene transfer and DNA binding protein experiments will be used to meet these qoals.

Progress: 87/01 to 87/12. The long-range goal of this project is to describe the molecular processes regulating soybean seed protein gene expression. My experiments have focused on the lectin gene because there is only one functional copy in the soybean genome, and because by laboratory has accumulated a large amount of information on lectin gene structure, organization, and regulation. In situ hybridization studies were used to describe the cell-specific lectin gene expression patterns during soybean development. Lectin mRNA accumulates in a "wave-like" manner from the exterior to the interior of the cotyledon during embryogenesis. This program is maintained in transformed tobacco plants. In addition, lectin mRNA is 200-fold less prevalent in the embryonic axis, and is localized in specific cell types of the germinating root. Deletion and DNA binding protein studies were initiated to identify cis-control elements and trans-acting regulatory molecules that control lectin gene expression. A DNA binding protein was identified that interacts with specific sequences in the lectin gene 5' region, and with other seed protein genes. Deletion studies in transformed tobacco plants showed that a cis-control element located > 0.5 kb from the 5' gene end is required for elevated embryo expression levels; however, only 80 base pairs contiguous to the 5' gene end is required for embryo-specific gene expression. The lectin DNA binding protein does not detectably bind with either the > 0.5 kb "enhancer" region or the 0.08 kb "developmental-specific" region.

Publications: 87/01 to 87/12

OKAMURO, J.K., JDFUKU, K.D. and GOLDBERG, R.B. 1986. Soybean seed lectin gene and flanking nonseed lectin genes are developmentally regulated in transformed tobacco plants. Proc. Nat. Acad. Sci. USA 83, 8240-8244.

JDFUKU, K.D., DKAMURO, J.K. and GDLDBERG, R.B. 1987. Interaction of an embryo DNA binding protein with a soybean lectin gene upstream region. Nature 328, 734-737.

BARKER, S.J., HARADA, J.J. and GDLDBERG, R.B. 1987. Cellular localization of soybean storage protein mRNA in transformed tobacco seeds. Proc. Nat. Acad. USA 84, in press.

23.002 CRISO131223
MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN
PSEUDOMONAS SYRINGAE PV. GLYCINEA

STASKAWICZ B J; Plant Pathology; University of California, Berkeley, **CALIFORNIA** 94720. Proj. No.: CA-B*-PPA-4786-CG Project Type: CRGO Agency ID: CRGO Period: O1 SEP 86 to 30 SEP 88

Objectives: PROJECT 8600299. The major objectives of this research are to determine the nucleotide sequence of the avirulence genes avrA, avrB and avrC and to study the expression of these genes both in vitro and in planta.

Approach: The determination of the primary nucleotide sequences will allow us to construct precise beta-galactosidase fusions. The fusion proteins will then be used to raise antisera and to analyze the expression of this protein both in vitro and in planta. In addition, we will attempt to localize the avirulence protein in subcellular fractions employing western blot procedures. The ultimate goal of this project is to determine the molecular basis of specificity and the induction of disease resistance in the bacterial blight disease of soybean.

Progress: 86/09 to 88/09. Pseudomonas syringae pv. glycinea is the causal agent of bacterial blight disease of soybean. The expression of disease resistance in the cultivar Harosoy is dependent on the resistance gene Rpgl and the presence of the avirulence gene avrB in Psg RO. In addition, the avirulence gene avrB is induced during the expression of disease resistance, while the bacterium is growing in the host. Experiments have been performed that have characterized the promoter region of avrB. The initiation of transcription has been determined by primer extension and has been identified to be 78 bp upstream from the initiation of translation. Finally, mutations in the hrp gene cluster have been identified that regulate \bar{t} he induction of

Publications: 86/09 to 88/09

TAMAKI, S., D. KOBAYASHI, B. STASKAWICZ, and N. T. KEEN. (1988). Construction of recombinant genes from avrB and avrC in order to localize regions of plant recognition specificity. Ann. Symp. Plant Path., Univ. of California, Riverside.

TAMAKI, S., D. DAHLBECK, B. STASKAWICZ and N. T. KEEN. (1988). Characterization and expression of two avirulence genes cloned from Pseudomonas syringae pv. glycinea. J. Bact. 170:4846.

23.003 CRISO033872 REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION

GDLDBERG R B; Regents of The University; University of California, Los Angeles, CALIFORNIA 90024.

Proj. No.: 8400679 Project Type: CRGD Agency ID: CRGD Period: 01 AUG 82 to 30 JUN 86 Objectives: Proj. No. 8200391. I propose to investigate the organization and expression of Kunitz trypsin inhibitor genes in normal (Ti+) and defective (Ti-) soybean lines. Ti- lines lack physiologically significant levels of Kunitz trypsin inhibitor. Three sprcific questions will be addressed in this project. First, at what cellular level is Kunitz trypsin inhibitor gene expressin blocked in Ti- plants? Second, what is the organizational pattern of Kunitz trypsin inhibitor genes in Ti+ chromosomes.

Approach: To answer these questions I will carry out restriction mapping, DNA hybridization, and electron microscopic studies using Kunitz trypsin inhibitor cDNA and genomic clones. The significance of this project is that it should yield new insight into the organization and expression of an agriculturally significant plant gene family.

Progress: 84/01 to 84/12. A Ti-plasmid vector was used to transform tobacco plants with soybean lectin and Kunitz trypsin inhibitor genes. Molecular and genetic analysis of the transformants indicated that these genes are represented once per transformed tobacco genome. Gel blot studies with seed mRNAs from different stages of development indicated that the soybean messages accumulate and decay in tobacco development similar to that which occurs in soybean. Experiments with tobacco leaf, root, and stem mRNAs indicated that the lectin and Kunitz trypsin inhibitor genes are expressed in the same transformed plant organ systems as in soybean. For example, the Kunitz trypsin inhibitor gene is expressed in tobacco leaf and stem while the lectin gene is not detectably expressed in these organ systems. In addition, three non-seed protein genes flanking the lectin gene are also expressed normally in tobacco indicating that a cluster of four closely-linked genes is corrected expressed in a foreign cell environment. These studies show that soybean seed protein genes contain cis-acting sequences which regulate their developmental-specific expression and that these sequences are recognized by trans-acting factors produced in tobacco cells. The implication of these findings to crop improvement is that molecular genetic procedures can be used to transfer large polygenic clusters from one plant to another and that the foreign genes will be expressed in the transformed plant.

Publications: 84/01 to 84/12
OKAMURO, J.K. and GOLDBERG, R.B. 1985.
Tobacco single-copy DNA is highly
homologous to sequences present in the
genomes of its diploid progenitors. Mol.
Gen. Genet. 198, 290-298.
WALLING, L., DREWS, G.N. and GOLDBERG, R.B.
1985. Transcriptional and
post-transcriptional regulation of soybean
seed protein mRNA levels. Proc. Nat. Acad.
Sci. USA IN PRESS.

23.004 CRISO130203
DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING
COMPLEX GENES

WALLING L L; Botany & Plant Sciences; University of California, Riverside, CALIFORNIA 92521.

Proj. No.: CA-R*-BPS-4779-CG Project Type: CRGO Agency ID: CRGO Period: 15 SEP 86 to 31 MAR 89

Objectives: PROJ 8601502. Investigate the developmental regulation of the soybean light-harvesting complex (LHCP) genes.

Approach: Isolate all of the soybean LHCP genes. Identify gene specific probes. Using gene specific probes, analyze the expression program for each LHCP gene. Determine the fine structure of the LHCP genes by DNA sequencing and S1 nuclease mapping. Initiate experiments to introduce the LHCP genes into tobacco. Initiate experiments to localize developmentally important regulatory signals.

Progress: 88/01 to 88/12. The soybean chlorophyll a/b (Cab) binding protein gene family consists of ten members. We have isolated nine of the ten soybean Cab genes. DNA sequence analysis of eight Cab genes have been completed. We have utilized Cab 1-5 DNA sequence data to establish (1) Cab 1 is a pseudogene, (2) Cab 2 may encode a 24 kD Cab protein of unusual structure, (3) Cab 3-5 and Cab 7-9 encode the major PSII 25 kD Cab proteins, (4) a soybean Cab phylogenetic tree, and (5) a Cab interspecies phylogenetic tree. We have recently determined Cab 7-9 sequences and will incorporate these data into our future phylogenetic analyses. Cab 1-5 DNA sequence data was used to develop DNA probes for monitoring individual Cab gene expression by \$1 nuclease protection assays. We have determined that during soybean development (1) Cab 1 and 2 mRNAs do not accumulate, (2) Cab 3-5 mRNAs are differentially regulated, and (3) Cab mRNAs decline in response to exogenous abscisic acid. We have quantitated the amount of total Cab and individual Cab RNAs in the polysomal (A+) mRNA population. Finally, we are determining the spatial distribution of Cab mRNAs by in situ hybridization. We have established these procedures in my laboratory and the first hybridization experiments indicate that waves of gene expression in the germinating cotyledons exist.

Publications: 88/01 to 88/12

WALLING, L.L., CHANG, Y.C., DEMMIN, D.S. and HOLZER, F.M. (1988). Isolation, characterization and evolutionary relatedness of three members from the soybean multigene family encoding chlorophyll a/b binding proteins. Nuc. Acids Res. 16:.

DEMMIN, D.S., STOCKINGER, E.J., CHANG, Y.C. and WALLING, L.L. (1989). Phylogenetic relationships between the chlorophyll a/b binding protein (Cab) multigene family: An intra- and inter-species study. J. Molec.

Evol. In press.

23.005* CRISO014667
MECHANISMS OF PATHOGENESIS AND RESISTANCE IN
PLANT-PARASITE INTERACTIONS

KEEN N T; SIMS J J; ENDO R M; Plant Pathology; University of California, Riverside, CALIFORNIA 92521.

Proj. No.: CA-R*-PPA-0865-H Project Type: HATCH Agency ID: CSRS Period: O3 OCT 85 to 30 SEP 90

Objectives: Elucidate the molecular mechanisms underlying the expression of single gene disease resistance in plants.

Approach: Screen libraries of Pseudomonas syringae pv. glycinea races for avirulence genes. Characterize genes by sequencing and translation into proteins in E. coli. Protein products will be isolated and used in assays to determine if they are the recognitional elements that interact with soybean resistance gene products. Using I labelling of the pathogen protein, isolate plant resistance gene proteins. Use plant proteins as antigens for antiserum production, which could be used to isolate the plant disease resistance gene from a phage library of plant DNA.

Progress: 88/01 to 88/12. The complete structure of four pel genes encoding pectate lyases from Erwinia chrysanthemi was published. Work was also completed on sequencing a new pectate lyase gene from Erwinia carotovora and this work is now in press and pending publication. Significantly, the new gene and a related gene from Yersinia pseudotuberculosis are considerably different from the pel genes of ${\sf E.}$ chrysanthemi. In other work, the structures of two additional avirulence (avr) genes was published during the year and the sequencing of an additional gene, avrD, has been completed. This latter gene has been shown to function in Escherichia coli cells to cause them to elicit the hypersensitive reaction in the proper soybean cultivars. Furthermore, culture fluids of the E. coli cells have been shown to contain a low molecular weight elicitor that also elicits the HR in these soybean cultivars. In collaborative work with Dr. Mark Stayton at the University of Wyoming, we are attempting to deduce the structure of this elicitor. It is believed that the information will give significant indications regarding the function of avrD in pseudomonads from which it has been cloned. The work also encourages us to attempt the cloning of the plant disease resistance gene complementing avrD.

Publications: 88/01 to 88/12

STASKAWICZ, B., DAHLBECK, D., KEEN, N.T. and NAPOLI, C. (1987). Molecular characterization of cloned avirulence genes from race O and race 1 of Pseudomonas syringae pv. glycinea. J. Bacteriol. 169:5789-5794.

SCHLEMMER, A.F., WARE, C.F. and KEEN, N.T. (1987). Purification and characterization of a pectin lyase produced by Pseudomonas fluorescence W51. J. Bacteriol. 169:4495-4498.

MANULIS, S., KOBAYASHI, D.Y. and KEEN, N.T. (1988). Molecular cloning and sequencing of a pectate lyase gene from Yersinia

pseudotuberculosis. J. Bacteriol. 170:1825-1830.

23.006* CRISO089829 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT

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PRING D R; CHOUREY P S; HIEBERT E; Plant Pathology; University of Florida, Gainesville, FLORIDA 32611.

Proj. No.: FLA-PLP-02317 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 82 to 30 SEP 88

Objectives: Identification and Characterization of Agriculturally Important Genetic Systems. Regulation of Gene Expression and The Delivery of Genetic Material to Higher Plants and Associative Microorganisms. Somatic Cell Genetics and Plant Development: The Modification, Selection Regulation, and Propagation of Plants through Cell and Tissue Culture.

Approach: Plant viral genomes will be mapped and their products characterized. Recognition factors, both host and bacterial, will be characterized. Transposable elements of maize will be used as specific mutagens to identify rate limiting steps in starch biosynthesis. Plasmid-like DNAs in maize and sorghum will be tested as genetic vectors. Plant regeneration from protoplasts and callus will be attempted via organogenesis and somatic embryogenesis.

Progress: 83/10 to 88/09. Promoters and processing sites of maize mitochondrial atp6 are positioned 5' to Turf-13 and ORF221 in T cytoplasm maize, providing regulatory sequences associated with the 13 kD gene product of Turf-13. Maize nuclear backgrounds influence abundance of at least five transcripts associated with the gene. Abundance of the 13 kD gene product is reduced dramatically, while the Rf1 restorer only slightly reduces abundance of major transcripts, suggesting a role of the gene in translation. A maize cell suspension culture was used to study the biology and replication of mitochondrial DNA (mt DNA) and the two minicircular DNAs. All mt DNAs were synthesized rapidly during logarithmic growth phase, whereas no synthesis could be detected in stationary phase. The minicircular DNAs replicated earlier than the principal mt DNA. These data indicate that components of mitochondrial genome exhibit differential replication. Restriction digestion and Southern blot analyses of the bean golden mosaic virus (BGMV) isolated in Florida in comparisons with the Puerto Rican BGMV isolate revealed a high degree of sequence homology between the two isolates. However, distinct restriction patterns with four different endonucleases indicate that the isolates are not identical at the genomic level. A monoclonal antibody prepared to the Florida BGMV was useful in distinguishing some biological variants of BGMV.

Publications: 83/10 to 88/09
KENNELL, J.C., WISE, R.P. and PRING, D.R.
 Influence of nuclear background on
 transcription of a maize mitochondrial

region associated with Texas male sterile cytoplasm. Mol. Gen. Genet. 210:399-406. 1987.

PRING, D.R., GENGENBACH, B.G. and WISE, R.P. Recombination is associated with polymorphism of the mitochondrial genomes of maize and sorghum. Phil. Trans. Royal Soc. London B 319:187-198. 1988.

SMITH, A.G., CHOUREY, P.S. and PRING, D.R. Replication and amplification of the small mitochondrial DNAs in a cells suspension of Black Mexican Sweet maize. Plant Molec. Biol. 10: 83-90. 1987.

GILBERTSON, R.L., FARIA, J.C., HIEBERT, E. and MAXWELL, D.P. 1988. Properties and cytology of bean golden mosaic in Brazil. Phytopathology, Abst. 441 submitted for the Annual Meetings of APS, San Diego, CA, Nov. 13-17, 1988.

CHANG, C.A., HIEBERT, E. and PURCIFULL, D.E. 1988. Characterization and immunological analysis of nuclear inclusions induced by bean yellow mosaic and clover yellow vein potyvirusus. Phytopathology 78, in press.

CHANG, C.A., HIEBERT, E. and PURCIFULL, D.E. 1988. Analysis of in vitro translation of the bean yellow mosaic virus RNA and the inhibition of proteolytic processing by antiserum to the 49K nuclear inclusion protein. J. gen. Virol.

HIEBERT, E. and DOUGHERTY, W.G. 1988.
Organization and expression of the vira.

23.007 CRISO096978 EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS

MEAGHER R; Research Foundation; University of Georgia, Athens, **GEORGIA** 30602.

Proj. No.: GEO-8502158 Project Type: CRGO Agency ID: CRGO Period: 15 SEP 85 to 15 SEP 87

Objectives: Proj. 8502158. There is considerable evidence that some plant actin genes are differentially expressed in different plant tissues. We propose to identify and characterize actin transcriptional control sequences in transgenic petunia plants.

Approach: Several of the soybean actin genes and appropriate chimeric derivative will be molecularly cloned into petunia leaf discs using Agrobacterium Ti plasmid technology. Transformed plants will be selected and regenerated from these leaf discs. Standard techniques of molecular biology will be used to characterize the copy number, physical structure, steady state levels of mRNA expression and transcription rates of these genes.

Progress: 87/01 to 87/12. We have investigated Petunia hybrida V23 and R51 as a genetic system in which to study the expression of soybean actin and Rubisco small subunit genes (SSU). First we characterized the transcription and steady state levels of expression of two petunia SSU genes and determined that their behavior is similar but not identical to that found for soybean genes in soybean. Both soybean and petunia SSU transcription is light and phytochrome

regulated and can be shut off in fully light grown plants with a few minutes of far red light. Transgenic constructs containing the 5' end of the soybean SSU genes SRS1 and SRS4, were fused to a bacterial kanamycin resistance gene. The SSU chimera followed all the patterns of expression expected for the SSU genes from etiher petunia or soybean. In conducting the control experiments to characterize the petunia system we discovered that the levels of petunia SSU RNA are controlled by a light inducedsystem of RNA turnover as is SSU RNA in soybean. This exciting new discovery is the focus of ongoing research and opens up a novel area of gene control not yet examined in plants. A genetic characterization of the petunia actin genes has opened up a new approach to estimating physical distancesin large plant genomes which should be applicable to most higher plant systems.

Publications: 87/01 to 87/12

BERRY-LOW, S. and MEAGHER, R.B. 1985.

Transcriptional Regulation of a Gene
Encoding the Small Subunit of Ribulose
Bisphosphate Carboxylase in Soybean is
Linked to the Phytochrome Response. J. Mol.
Cell. Biol. 5:1910-1917.

GRANDBASTIEN, M.A., BERRY-LOWE, S. and
MEAGHER, R.B. 1986. Two Soybean
Ribulose-1,5-Biphosphate Carboxylase Small
Subuunit Genes Share Extensive Homology
Even in Distant Flanking Sequences. Journal
Plant Mol. Biol. 7:451-465.

SHIRLEY, B.W., BERRY-LOWE, S.L., ROGERS,
S.G., FLICK, J.S., HORSCH, R., FRALEY, R.T.
and MEAGHER, R.B. 1987.

23.008 SOYBEAN BROWN STEM ROT

CRISO141585

GRAY L E; Agricultural Research Service,
Urbana, ILLINOIS 61801.
Proj. No.: 3611-24000-001-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 APR 86 to 31 MAR 91

Objectives: Establish the underlying mechanisms controlling pathogenicity of isolates of Phialophora and ascertain how environmental factors influence pathogenesis.

Approach: 1) Collect isolates of Phialophora from geographical areas and determine pathogenicity of isolates on soybeans. 2) Bioassay culture filtrates of Phialophora isolates against soybean callus and soybean cell suspension cultures to ascertain if a toxin is involved in pathogenesis. 3) Determine the role of air temperatures in pathogenesis and host respose to infection. Determine how temperature influences fungus sporulation in the host plant and development of the fungus in the leaf vascular tissue. 4) Establish environmental effects on toxin production in culture.

Progress: 88/01 to 88/12. Ten soybean Phialophora gregata isolataes were obtained from 4 midwest states. Total genomic DNA was prepared from each isolate. In addition genomic DNA was prepared from 5 Phialophora isolates that are pathogenic to adzuki bean. Random DNA

fragments from a virulent isolate of Phialophora were cloned into E. coli to generate a number of random probes. Restriction fragment length polymorphism between soybean and adzuki bean Phialophora isolates is currently being determined. To date, based on a limited number of probes, considerable variation in fragment length patterns has been detected between the soybean and adzuki bean fungus isolates and some variation has been detected between soybean isolates. A soybean tissue culture assay was recently developed that can be used to assay metabolites produced by isolates of Phialophora. This new tissue assay has completely eliminated solvent problems that were inherient in previous assay systems. Amber-blue Fusarium solani isolates were recovered from soybean plants with Soybean Sudden Death Symptoms in 1988. These isolates were used to inoculate sovbean plants in the greenhouse and are being evaluated for pathogenicity and their role in causing SDS symptoms on plants.

Publications: 88/01 to 88/12
GRAY, L.E. 1988. Development of Phialophora gregata in soybean plants. Phytopathology Abstract 78:1502.
MONTE-NESHICH, D.D., GRAY, L.E. and HEPBURN, A. 1988. Responses of soybean cells to culture filtrate of Phialophora gregata. Phytopathology Abstract 78:1503.
HANSON, P.M., NICKELL, C.D., GRAY, L.E. and SEBASTIAN, S. 1988. Identification of two dominant genes conditioning brown stem rot resistance in soybean. Crop Science 28:41-43.

23.009 CRISO049544 NEW GENETIC METHODS FOR IMPROVING SOYBEANS

TBD; Photosynthesis Research Unit; Agricultural Research Service, Urbana, **ILLINOIS** 61801.

Proj. No.: 3611-20170-010-00D

Project Type: INHOUSE Agency ID: ARS Period: 01 OCT 84 to 31 MAR 86

Objectives: Devise new genetic methodilogies for modifying genes and their expressions in metabolic processes involved in yield and product quality of soybeans. Studies will include nuclear and extranuclear, genomic, and biochemical analyses to identify and isolate specific genes and combinations of genes which regulate reproduction, growth, and development. This is a new start which will be funded with FY85 Budget Increase Money. P. Miller is NPS contact. Money is to be added from another source.

Approach: Pending more information.

Progress: 86/01 to 86/03. I. Transcription Initiation: The spinach atp8 gene has three putative promoter regions at positions 455, 275, and 180 base pairs before the protein-coding region. We observe that on a recombinant DNA containing all three promoter-like sequences, transcription initiation occurs primarily at the 455 position, and to a lesser extent at the 275 and

180 positions. On a recombinant DNA lacking the 455 region, transcription initiation is nonspecific. However, a recombinant DNA that lacks both the 455 and 275 regions is capable of promoting specific transcription initiation in the 180 region. II. Transcription Termination: A 226 base pair DNA fragment containing the 5' end of the rbcL gene was cloned into the vector pUC13, adjacent to a 280 base pair DNA fragment containing the 3' end of this gene. The resulting recombinant DNA contains a 220 base pair rbcL "minigene", which lacks most of the rbcL protein-coding region. This minigene construction will be used to study transcription termination in vitro. III. Rubisco Mutagenesis: A restriction DNA fragment containing the pea rbcL gene was digested with Bal 31 exonuclease and cloned into the T7 expression vector pTZ18. The goal was to remove all the 5' DNA sequence that precedes the pea rbcL protein coding region, except for the ribosomebinding site, located eight base pairs before the initiator codon. Using restriction enzyme and DNA sequencing analyses, the appropriate clone was selected which contains the rbcL protein-coding region and only nine base pairs of 5' "leader" sequence.

Publications: 86/01 to 86/03
OROZCO, E.M. JR., MULLET, J.E.,
HANLEY-BOWDOIN, L. and CHUA, N._H. 1986. In
vitro transcription chloroplast protein
genes. Methods Enzymol. 118:232-253.

23.010* CRISO095743 ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS

HEPBURN A G; Agronomy; 1301 West Gregory
Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-15-0363 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To isolate agronomically important genes of known function from crop species or from species related to crops species. To insert known autotransposing elements into plant species showing agronomically significant characters and to screen for transposon mutagenesis of those characters.

Approach: Bacteriophage Lambda expressing libraries will be made of cDNA from plant species and screened using antibodies raised against important proteins. To induce transposon mutagenesis, a known plant transposable element (Mu, Ac or Tam 3) will be inserted into the host species genome using disabled Agrobacterium vectors and the resultant tissue or regenerated plants screened for evidence of transposon inactivation of specific characters.

Progress: 87/10 to 88/09. The induction of genes in soybean plants cultured cells in response to culture filtrate of the pathogenic fungus Phialophora gregata has been studied. cDNA libraries have been produced and ten clones selected that are induced in resistant plants following toxin exposure. These clones isolated originally in bacteriophage lambda gt10 have been recloned into a pasmid vector.

The clones have been used as probes against mRNA preparations isolated from resistant and sensitive cultivars of soybean innoculated with the fungus. The alterations in the expression of the genes corresponding to the clones has been studied with a view to identifying genes that may correspond to the primary resistance response. A clone of the soybean chalcone synthase gene was used as a control. Clone G10-1 was the only clone which showed specific induction in the leaves of resistant plants treated with the fungus. Clone G5-2 was induced in stems of either susceptible plants (Corsoy) inoculated with a nonpathogenic fungal isolate (It) or resistant plants (PI437833) inoculated with a pathogenic isolate (C4). Each of these represents a nonproductive infection and hence this clone may be involved in the response to fungal attack. As part of our studies to isolate and identify important genes in crop species, we have also been examining the DNA transfer capability of Agrobacterium tumefaciens with a view to developing more efficient transformation vectors. It is hoped that such vectors will help to overcome the low DNA transfer efficiency from A.

MONTE-NESHICH, D. C. Molecular reponse of soybean (Glycine max (L.)) to Phialophora gregata. Ph.D. Thesis, University of Illinois at Urbana-Champaign, (1988).
CULIANEZ-MACIA, F. C. and HEPBURN, A. G. (1988) Right-border sequences enable the left border of an Agrobacterium tumefaciens nopaline Ti-plasmid to produce single-stranded DNA. Plant Molecular Biology 11:389-399.
CULIANEZ-MACIA, F. C. and HEPBURN, A. G. (1988) The kinetics of T-strand production in a nopaline-type helper strain of Agrobacterium tumefaciens. Molecular Plant-Miscrobe Interactions 1:207-214.

Publications: 87/10 to 88/09

23.011 CRISO133914
STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSERTION SEQUENCE

VODKIN L 0; Agronomy; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.

Proj. No.: ILLU-15-0513 Project Type: CRGO Agency ID: CRGO Period: 01 SEP 87 to 31 AUG 88

Objectives: PROJ. 8702811. To determine the structure of a 3.4 kb insertion element which interrupts a soybean lectin gene and to examine the nature of sequences related to this element which are present elsewhere in the soybean genome. The lectin gene insertion has certain features analogous to gransposable element.

Approach: The complete nucleotide sequence of the lectin gene insertion element will be determined using Bal-31 nuclease an create an overlapping set of deletion fragments which are subcloned into the single-stranded M13 phage vectors and sequenced by the dideoxy method. Synthetic primers will be used to overlap certain regions if necessary. Potential open reading frame regions within the insertion element will be cloned into expression vectors. Genomic regions harboring sequences related to

the lectin insertion element will be isolated from soybean libraries and characterized by restriction mapping and sequencing where appropriate.

Progress: 87/10 to 88/09. We have compared the organization of six Tgm elements that were selected from a genomic library of sovbean DNA on the basis of hybridization with subcloned regions of Tgm1 (transposon, Glycine max) from the seed lectin gene. These elements ranged in size from 1.6 kilobase pairs to greater than 12 kilobase pairs. Tgm2, Tgm3, Tgm4 and Tgm5 represent partial isolates in which the genomic clone contained a 3' but not a 5' terminus of the element; while Tgm6 and Tgm7, like Tgm1, were small isolates flanked by both 5' and 3' non-element sequences. Cross-hybridization studies between subcloned portions of these seven elements identified regions of homology which suggest that the Tgm transposable elements of soybean form a family of deletion derivatives. In addition to internal deletion events, numerous deletions and base substitutions are also present within the borders of these elements which are comprised of the same tandemly repeated sequence. The 39% amino acid homology between a 1 kilobase portion of an open reading frame in Tgm4 and Tgm5 and ORF1, an open frame from the first intron of the maize Enhancer (Suppressor-mutator) transposable element, suggests that both elements encode a common function that requires a high degree of protein conservation.

Publications: 87/10 to 88/09
RHODES, P. R. and VODKIN, L. O. (1988).
 Organization of the Tgm family of
 transposable elements in soybean. Genetics
120: 597-604.

23.012 CRISO141578
MANIPULATION OF STORAGE PROTEIN STRUCTURE IN
SOYBEANS

NIELSEN N C; Agricultural Research Service, West Lafayette, INDIANA 47907. Proj. No.: 3602-22230-001-00D

Project Type: INHOUSE Agency ID: ARS Period: 01 APR 86 to 31 MAR 91

Objectives: 1) Identify linkage relationships among/between glycinin and B-conglycinin genes.
2) Determine molecular basis of seed protein mutations. 3) Deter- mine regions in subunits amenable to modification by site-directed mutagenesis. 4) Identify enzymes involved in maturation of storgae proteins.

Approach: Identify seed protein variants by changes in electrophoretic mobility, and restriction fragment length polymorphisms flanking or in glycinin and B-conglycinin genes. Use oligonucleotide-directed mutagenesis to alter conserved cleavage site between acidic and basic components of glycinin. Insert cleavage site in B-conglycinin at the analogous position as glycinin. Express modified coding regions in an in-vitro system, in yeast, or in a higher plant. Test effect of mutations on assembly of storage protein complex. Identify

enzymes involved in glycinin maturation with proglycinin made in vitro as substrate. West Lafayette, IN, Lilley Hall; Rm 2-330, 2-331, 2-332: BL-2; Sep 29, 1980.

Progress: 88/01 to 88/12. Glycinin, a predominant seed storage protein in soybeans, undergoes a complex series of post-translational events. Precursor subunits are synthesized in endoplasmic reticulum and then aggregate into trimers. The trimers move through the endomembrane system to protein bodies where they aggregate into an insoluble form. Associated with the aggregation is a post-translational cleavage at a highly conserved site. The cleavage yields mature subunits that are organized into trimers and consist of two peptide chains that are covalently linked via a disulfide bond. By testing the ability of precursors to be re-assembled into hexamers along with mature dissociated glycinin subunits, the post-translational cleavage has been shown to be required for assembly beyond the trimer stage. These findings are considered to show that the cleavage reflects a regulatory step that keeps the subunits from aggregating and precipitating into an insoluble form prior to reaching their final site of deposition.

Publications: 88/01 to 88/12

NIELSEN, N.C. and WILCOX, J.R. 1988.

Biotechnology for soybean improve- ment.

IN: World Conference on Biotechnology for the Fats and Oils Industry. American Oil Chemists' Society, Urbana. pp. 58-64.

DICKINSON, C.D. EVANS, R.P. and NIE SEN, N.C. 1988. RY-repeats are conserved in the 5'-flanking regions of legume seed protein genes. Nucleic Acids Research 16:371.

NIELSEN, N.C. 1988. IN VITRO modification and assembly of soybean glycinin. IN: Proc. World Cong. and Expo on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs. (Willhoite & Beery, eds.) Accepted October 3, 1988.

23.013 CRISO076310 SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS

NIELSEN N C; Agronomy; Purdue University, West Lafayette, **INDIANA** 47907. Proj. No.: INDO50074 Project Type: STATE

Agency ID: SAES Period: 01 OCT 84 to 30 SEP 89

Objectives: To identify structural genes for prevalent seed proteins in the soybean genome. To study their structure and the events which

prevalent seed proteins in the soybean genome. To study their structure and the events which occur during expression. To identify the structural features of glycinin required for complex assembly and which modulates its nutritional and functional properties. To characterize leaf storage glycopeptides and their function during pod fill. To evaluate the effect of lipoxygenase null-alleles and low 18:3 genes in flavor stability of soybeans and soy products.

Approach: Glycinin and B-conglycinin genes will be purified and characterized with regard to structure, inheritance, linkage and and factors

that modulate expression. Precursors made in vitro will be used to study the steps involved in post-translational modification and their consequence on structure. Site directed mutagenesis will be used to evaluate opportunities to manipulate nutritional and functional properties of glycinin. The leaf storage proteins will be uprified and and characterized. The lipoxygenase null-alleles and/or low 18:3 genes will be introduced into common genetic backgrounds and their effect on flavor stability compared with seeds from near-isogenic controls.

Progress: 87/10 to 88/09. Glycinin, a predominant seed storage protein in soybeans, undergoes a complex series of post-translational events. Precursor subunits are synthesized in endoplasmic reticulum and then aggregate into trimers. The trimers move through the endomembrane system to protein bodies where they aggregate into an insoluble form. Associated with the aggregation is a post-translational cleavage at a highly conserved site. The cleavage yields mature subunits that are organized into trimers and consist of two peptide chains that are covalently linked via a disulfide bond. By testing the ability of precursors to be re-assembled into hexamers along with mature dissociated glycinin subunits, the post-translational cleavage has been shown to be required for assembly beyond the trimer stage. These findings are considered to show that the cleavage reflects a regulatory step that keeps the subunits from aggregating and precipitating into an insoluble form prior reaching their final site of deposition.

Publications: 87/10 to 88/09
DICKINSON, C. D., FLOENER, L. A., LILLEY, G.
A. and NIELSEN, N. C. (1987) Self-assembly
of proglycinin and hybrid proglycinin
synthesized in vitro from cDNA. Proc. Natl.
Acad. Sci. USA 84:5525-5529.

DAVIES, C. S., CHO, T. J. and NIELSEN, N. C. (1987) Tests for linkage of seed protein genes Lx1, Lx3, and Cgy1 to markers on known linkage groups. Soybean Genetics Newsletter, 14:248-252.

DAVIES, C. S., NIELSEN, S. S. and NIELSEN, N. C. (1987) Flavor improvement of soybean preparations by genetic removal of lipoxygenase. J. Am. Oil Chem, Soc. 64:1428-1433.

23.014 CRISO140762 CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS

PALMER R G; SHOEMAKER R; Agricultural Research Service, Ames, **IOWA** 50010. Proj. No.: 3625-21000-001-00D

Project Type: INHOUSE Agency ID: ARS Period: 01 OCT 85 to 30 SEP 90

Objectives: To construct a chromosomal linkage map in soybean using restriction fragment length polymorphisms with DNA markers.

Approach: Hand pollination, seed increase, and freeze drying of leaf samples will be done in Iowa and Puerto Rico. Work done in Utah will include extraction of DNA from leaf tissue and examination of the material on Southern blots for polymorphisms using a number of restriction enzymes. A genomic library of soybean will be screened to isolate random clines which represent single orlow copy sequences. These will be used as probes in the Southern blotting experiments to determine whether they can serve as markers to distinguish between the two parental varieties. Once a collection of markers has been obtained, the markers will be mapped with respect to one another and to other morphological plant traits by examining their segregation patterns in backcrossed plant material. Additionally, a somatic approach to mappingmay be undertaken by examining the behavior of markers during chromosome loss induced in tissue culture.

Progress: 88/01 to 88/12. Polyacrylamide gel electrophoresis detected superoxide dismutase (SOD) activity in soybean seed. Inheritance indicated a single locus with codominant alleles (Sod 2-s) and (Sod 2-6). No linkage was detected between the Sod 2 locus and 12 other loci tested. Three F2 populations segregating for isocitrate dehydrogenase (Idhl) and non-nodulating root (rj1) had recombination values between 24.6 to 30.9%. Three F2 populations segregating for isocitrate dehydrogenase and fasciated (f) stem had recombination values between 22.2 to 30.5%. F2 populations with all three loci indicated that the gene order was f-Idhl-rj1. These mutants are on LG11 in soybean. Fifty-eight accessions from the germplasm collection were screened with 17 molecular markers to assess diversity and allelic structure of the subgenus. Diversity was limited primarily to a 1 locus, 2 allele structure. Thirty-five percent of the polymorphisms were the result of rare alleles. F3 families of a cross between a G. max line and G. soja accession were evaluated for agronomic characters of morphology, maturity, and seed permeability. Molecular markers were identified which can account for much of the variation within these characters. Currently, 68 markers have been tested for linkage and 54 molecular markers, 1 isozyme, and 3 morphological markers have been linked. These linkages have been placed into 17 groups covering approximately 650 cu.

Publications: 88/01 to 88/12 KEIM, P., DIERS, B.W., PALMER, R.G., SHOEMAKER, R.C., MACALMA, T. and LARK, K.G. 1989. Mapping the soybean genome with RFLP markers. Proc. IV World Soybean Res. Conf. Accepted March 12, 1988. KEIM, P. and SHOEMAKER, R. 1988. Construction of a random recombinant DNA library that is primarily single-copy sequences. Soybean Genetic Newsletter 15:147-148. KEIM, P., OLSON, T. and SHOEMAKER, R. 1988. A rapid protocol for isolating soybean DNA. Soybean Genetic Newsletter 15:148-152. KEIM, P., SHOEMAKER, R.C. and PALMER, R.G. 1989. Restriction fragment length polymorphism diversity in soybean. Theoretical and Applied Genetics. Accepted Dec. 28, 1988.

23.015 CRISO041049 GENETICS AND CYTOGENETICS OF SOYBEANS

PALMER R G; COX T S; Bovine Paratuberculosis Res Bacteriology Laboratory; Agricultural Research Service, Ames, IOWA 50010. Proj. No.: 3808-20080-003-00D

Project Type: INHOUSE Agency ID: ARS Period: 20 MAY 74 to 20 MAY 86

Objectives: Investigate genetically and cytogenetically the nuclear and cytoplasmic genomes of soybeans, and close relatives of soybeans, so that the agronomic possibilities of soybeans can be enhanced and improved.

Approach: Nuclear mutants and cytoplasmic mutants will be analyzed genetically, chemically, electrophoretically and cytologically, by light-microscopy, fluorescence-microscopy and electron-microscopy, with a view to discovering the gene arrangement on the various chromosomes. Cytoplasmic mutants will be corssed with nuclear mutants in an attempt to discover possible cytoplasmic-nuclear interactions. Interspecific crosses will be generated between Glycine max and G. soja in an attempt to identify and characterize chromosome translocations. Meiotic mutants and polyploids will be screened for aneuploids. Trisomics and translocations will be identified and then crossed to the nine linkage groups to identify genes with respect to individual chromosomes. Mutants will be studied for their potential in achieving greater disease resistance and insect resistance and improving the protein (amino acid) content of the domestic soybean.

Progress: 85/01 to 85/10. Two cytoplasmically inherited foliar mutants were analyzed. One (cyt-Y3) was described genetically, ultrastructurally, and chemically. The second mutant was cyt -Y2. In the genotype cyt-Y2 y20-k2/y20-k2, plants are conditionally lethal. That is, the plants are lethal under field conditions but survive under greenhouse conditions. Maternal inheritance was shown for chloroplast DNA within the genus Glycine subgenus soja. Chromosome aneuploids, trisomic A, B, C, and D were studied morphologically. Statistical tests indicated that morphology was not a reliable variable to distinguish disomics from trisomics. The first chromosome interchange in soybeans was described agronomically and genetically. Seed yield among the three structural types was similar. Pollen and ovule was absorbed about 50% in heterozygous plants. A combination of Mayer's hemalum staining and methyl salicylate clearing was developed to analyze megasporogenesis and megagametogenesis. This technique will be used to study cytologically meiotic and post-meiotic mutants. Six source populations of the ms1 mutant were studied for frequencies of polyembryonic seedlings and different levels of polyploidy among abnormal seedlings. Variation was associated with the different ms1 alleles and was affected by other gene(s) and(or) environmental factors.

Publications: 85/01 to 85/10

- GWYN, J.J., PALMER, R.G. and SADANAGA, K. 1985. Morphological discrimination among some aneuploids in soybean (Glycine max (L.) Merr.). Canadian J. Genetics and Cytology 27:608-613.
- HATFIELD, P.M. SHOEMAKER, R.C. and PALMER, R.G. 1985. Maternal inheritance of chloroplast DNA within the genus Glycine subgenus soja. J. Heredity 76:373-374.
- STELLY, D.M. and PALMER, R.G. 1985. Relative development of basal, medial, and apical ovules in soybean. Crop Science 25:877-879.
- PALMER, R.G. and RDDRIQUEZ DE CIANZID, S. 1985. Conditional lethality involving nuclear and cytoplasmic chlorophyll mutants in soybeans. Theoretical and Applied Genetics 70:349-354.
- CHEN, L.F.D., HEER, H.E. and PALMER, R.G. 1985. The frequency of polyembryonic seedlings and polyploids from ms1 soybean. Theoretical and Applied Genetics 69:271-277.
- SHDEMAKER, R.C., CDDY, A.M. and PALMER, R.G. 1985. Characterization of a cytoplasmically inherited yellow foliar mutant (cyt-Y3) in soybean. Theoretical and Applied Genetics 69:279-284.
- PALMER, R.G. and HEER, H>E. 1984. Agronomic characteristics and genetics of a chromosome interchange in soybean. Euphytica 33:651-663.

23.016 CRISO140573 GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING

PALMER R G; SHDEMAKER R C; Cereal & Soybean Improvement Research Unit; Agricultural Research Service, Ames, **IOWA** 50010. Proj. No.: 3625-22000-001-00D

Project Type: INHDUSE Agency ID: ARS Period: 01 OCT 85 to 30 SEP 90

Objectives: 1) Identify 20 linkage groups; 2) identify and characterize aneuploids; 3) Identify chromosome interchanges; 4) Identify and characterize mutants; 6) Identify and characterize cytoplasmic mutants; 7) Develop and utilize electrophoretic techniques; 8) Develop techniques for rescuing unusual chromosome number plants.

Approach: Classical genetics combined with light and electron microscopy will allow us to study the inheritance and structural comparisons between normal and mutant. Electrophoretic techniques are used not only in inheritance studies but do provide some evidence for functional analyses. Tissue culture will provide us the means to rescure unusual chromosome types.

Progress: 88/01 to 88/12. Seven cytoplasmic groups were identified, based on ctDNA variation. Mutations were located to specific regions of the chloroplast chromosome. The effect of cytoplasm on carbon fixation efficiency was determined to be minimal among reciprocal F1s. Partial dominance for low CER was observed. Fatty acid compositions of maturing somatic embryos were found to be genotype dependent. Dver 500 soybean plants

were regenerated from tissue culture to evaluate the effect of passage through culture on transposable element activity. A hybrid plant from a G. \max x G. tomentella cross was induced to set seed. Biochemical and genetic factors suggest that elimination of perennial genome has occurred. The progeny of this cross are fertile and may serve as a 'bridge' by which to introduce genes from the gene pool of the perennials. A shrivelled-seed mutant was shown to have Mendellian inheritance and was associated with loss of major seed proteins. The frl locus (non-fluorescent root) was shown to be linked to the ep locus (low level seed coat peroxidase) with recombination about 41.4 cM. This linkage represents a new linkage group, LG12, in soybean. The morphological mutant, fasciated, was studied genetically and anatomically. The trait is inherited as a single-gene recessive. Fasication results from a gradual elaboration of the shoot apical meristem along one transverse axis; thus a conical apex becomes a generative ridge.

Publications: 88/01 to 88/12

GRAYBDSCH, R.A. and PALMER, R.G. 1988. Male sterility in soybean--An overview. American Journal of Botany 75:144-156.

- GRDDSE, R.W., WEIGELT, H.D. and PALMER, R.G. 1988. Somatic analysis of an unstable mutation for anthocyanin pigmentation in soybean. Journal of Heredity 79:263-267.
- LAMOTTE, C.G., CURRY, T.M., PALMER, R.G. and ALBERTSEN, M.C. 1988. Develop- mental anatomy and morphology of fasciation in soybean (Glycine max). Botanical Gazette. Accepted for Dec. 1988 Issue.
- DIETHELM, R., SHIBLES, R., GREEN, D. and SHDEMAKER, R. 1989. Influence of different cytoplasms on CO2-exchange rate, photosynthetic electron transport, and ... leaves. Crop Sci. March-April Issue.
- HDNEYCUTT, R., BURTDN, J., SHOEMAKER, R. and PALMER, R. 1989. Expression and inheritance of a shrivelled-seed mutant in soybean. Crop Sci. Accepted Nov. 4, 1988.

23.017 CRISOO93817 RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN

PALMER R G; FEHR W R; Agronomy; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: IOW02708 Project Type: SPECIAL GRANT Agency ID: CSRS Period: O1 SEP 84 to 31 AUG 87

Objectives: Isozyme loci are utilized in cultivar identification, in genetic linkage studies, as genetic markers in plant breeding or tissue culture, and in evolutionary studies. The primary objective of this research is to test for linkage of isozyme loci with chromosome interchanges and qualitative characters of soybean (Glycine max ?L.? Merr.).

Approach: Knowledge of the inheritance of alleles at isozyme loci, the linkage association between isozyme loci, and their linkage with qualitative characters would enhance conventional and molecular breeding approaches for soybean improvement. Only 13 of the 20 possible linkage groups in soybean have

been identified.

Progress: 84/09 to 87/08. Trisomic plants for chromosomes A, B, C, D, and S were assayed for their isozyme profiles. These trisomic plants were crossed to germplasm accessions that differed from the trisomics at as many isozymes as possible. A total of 14 different enzyme loci can be detected using techniques in our laboratory. Genetic segregation ratios of disomic plants (controls) were compared to genetic segregation ratios from trisomic plants. The analyses are not yet completed, but to date, the dia 1 (diaphorase) locus is located on the extra chromosome of Trisomic D.

Publications: 84/09 to 87/08

- GRIFFIN, J.D. 1986. Genetic and germplasm studies with several isoenzyme loci in soybean. Ph.D. Diss. Iowa State University, Ames, Iowa.
- GRIFFIN, J.D. and PALMER, R.G. 1987. Locating the Sp1 locus on soybean linkage group 1. Journal of Heredity 78:122-123.
- GRIFFIN, J.D. and PALMER, R.G. 1987.
 Inheritance and linkage studies with five isozyme loci in soybean. Crop Science (In Press).

23.018 CRISO098563 GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN

PALMER R G; SHDEMAKER R C; Agronomy; Iowa State University, Ames, **IOWA** 50011. Proj. No.: IDW02763 Project Type: STATE Agency ID: SAES Period: 01 MAY 86 to 30 JUN 90

Objectives: To investigate genetically, by using cytogenetics, tissue culture, and molecular biology, the nuclear and cytoplasmic genomes of soybeans and close relatives of soybeans, so that the agronomic potential of soybeans can be enhanced and improved.

Approach: Members of the genus Glycine, which includes cultigens, wild annuals, and wild perennial types, will be investigated by use of classical and modern genetic techniques. Nuclear and cytoplasmic mutants, and their interactions, will be studied to determine inheritance, linkage relationships, and developmental regulation. Chromosome aberrations, aneuploids, and interchanges will be used to identify linkage relationships. Restriction-fragment-length polymorphisms of nuclear DNA and organelle DNA will complement classical genetic mapping. Tissue culture and cell culture will be used to regenerate soybeans. Regenerated plants will be examined for variation. Tissue and cell culture will be used in interspecific crossing experiments and these hybrid products may aid in transferring traits between species or between genes.

Progress: 88/01 to 88/12. Dver 800 soybean plants have been regenerated from tissue culture. They are being evaluated for genetic variations. We have observed changes in isoenzymes and many morphological variants. A fertile hybrid has been recovered from a wide cross between G. max and G. tomentelle. Sterile

plants from this cross and progeny from the fertile cross are currently being studied to determine why one cross is fertile. Genetic transformation experiments are being carried out using particle acceleration technology. Transient expression of genes has been observed in calli and developing meristerns. Transformation technologies will allow for the transfer of genes from nearly any source, into soybean. The locations of various mutations causing restriction fragment length polymorphisms have been mapped to the chloroplast chromosome. Cultivars with diverse cytoplasms are being evaluated for the possible effect of cytoplasm on agronomic traits. If significant, cytoplasm will be considered in breeding programs. The fr1 locus (non-fluorescent root) was shown to be linked to the ep locus (low level seed coat peroxidase) with recombination about 41.4 centimorgans. This linkage represents a new linkage group, LG 12, in soybean, Polyacrylamide gel electrophoresis followed by specific staining detected superoxide dismutase (SDD) activity in soybean seed. The inheritance of variant patterns indicated a single locus with codominant alleles (Sod2-a), slow pattern and (Sod2-b), fast pattern.

Publications: 88/01 to 88/12

- R. A. GRAYBDSCH and R. G. PALMER. (1988). Male sterility in soybean--An overview. American Journal of Botany 75:144-156.
- R. W. GRDDSE, H. D. WEIGELT, and R. G. PALMER. (1988). Somatic analysis of an unstable mutation for anthocyanin pigmentation in soybean. Journal of Heredity 79:263-267. R. SHDEMAKER and E. HAMMDND. (1988). Fatty acid composition of soybean Glycine max (L.) Merr. somatic embryos. In Vitro 24:829-832.
- P. CLDSE, R. SHDEMAKER, and P. KEIM. (1989). Distribution of restriction site polymorphism within the chloroplast genome of the genus Glycine, subgenus Soja. Theor. Appl. Genet. (in press).
- R. DIETHELM, R. SHIBLES, D. GREEN, and R. SHDEMAKER. (1989). Influence of different cytoplasms on CD(subscript 2)-exchange rate, photosynthetic electron transport, and ribulose-1,5-bisphosphate carboxylase activity of soybean leaves.

23.019 CRISO074190 REGULATION OF PHOTOSYNTHETIC PROCESSES

SHIBLES R M; GREEN D E; Agronomy; Iowa State University, Ames, IOWA 50011. Proj. No.: IDW02275 Project Type: HATCH Agency ID: CSRS Period: 01 DCT 87 to 30 SEP 92

Objectives: Determine the roles of rubisco activase and 2-carboxyarabinatol-1-phosphate in the light-dark regulation of rubisco activity and photosynthesis. Determine the relationship of phloem unloading with photosynthetic rate.

Approach: Genetic lines of soybean qualitatively different in chloroplast DNA will be produced based upon RFLP patterns of soybean chloroplast DNA that have been identified. Rubisco activity and photosynthetic rates will

be measured in parents, reciprocal F(1)s, F(2) families, and the BC(6) isolines. Sink demand of soybean will be altered by various physical manipulations and environmental changes, and the sink effect on leaf photosynthesis will be investigated in relation to changes in rubisco content and activation ratio, inhibitor levels, and other metabolic regulators.

Progress: 88/01 to 88/12. Development of isogeneic lines of soybean that differ in chloroplast genome continues; F(subscript 5) plants will be available in 1989. Reciprocal F(subscript 1) crosses involving the same parents has revealed that Harosoy carries a cytoplasmic genome for rapid photosynthesis. However, it was not possible to identify the specific chloroplastic genes responsible for enhanced photosynthetic rate. Thinning the soybean plant stand at R2 to 25% of its normal population density increased pod set and pod plus seed growth rate per plant. The greater sink demand was paralleled by faster photosynthetic rates in three leaves (5, 7, and 10). Faster photosynthesis was a consequence of more Rubisco protein rather than greater Rubisco specific activity. The presence of young seeds on a soybean plant seems to have a retarding effect on the leaf's senescence. The onset of rapid seed growth delays onset of senescence in leaves that develop after seed growth begins, and it seems to arrest the decline in activities of leaves that recently initiated senescence. The mechanism of the seed effect has not been elucidated.

Publications: 88/01 to 88/12 FORD, D. M. AND SHIBLES, R. (1988). Photosynthesis and other traits in relation to chloroplast number during soybean leaf senescence. Plant Physiol. 86:108-111. DIETHELM, R. AND SHIBLES, R. (1989). Relationship of enhanced sink demand with photosynthesis and amount and activity of ribulose 1-5, bisphosphate carboxylase in bean leaves. J. Plant Physiol. (In press). DIETHELM, R., SHIBLES, R., GREEN, D. E., AND SHOEMAKER, R. C. (1989). Cytoplasmic effects on photosynthetic activities of soybean leaves. Crop Sci. (In press). SHIBLES, R., FORD, D. M., AND SECOR, J. (1989). Regulation of soybean leaf photosynthesis. In Proceedings of World Soybean Research Conference IV. Buenos Aires, Argentina, March 5-9, 1989. (In press).

23.020 CRISO136273 GENETIC ENGINEERING OF OILSEED SPECIES TO IMPROVE OIL QUALITY

WURTELE E S; NIKOLAU B J; HAMMOND E G; Food Technology; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: IOWO2893 Project Type: HATCH Agency ID: CSRS Period: 11 OCT 88 to 30 SEP 93

Objectives: Determine biochemical and molecular factors regulating triacylglycerol biosynthesis in developing oilseed embryos and characterize associated genes. Characterize DNA regulatory sequences which direct gene expression

specifically during embryo development and test these in transgenic plants.

Approach: Biochemical and molecular studies will be conducted to determine key regulatory steps in the biosynthesis of triacylglycerol. As regulatory enzymes are identified, the genes coding for these enzymes will be cloned, characterized, and appropriate gene constructions will be genetically engineered into oilseed species. The resulting transgenic plants will be tested for altered lipid production. DNA regulatory sequences from genes which we have already obtained which are developmentally regulated during embryo development will be characterized and tested for their ability to direct gene expression in transgenic plants. Such DNA sequences may be used to direct the expression of genes isolated in approach 1.

Progress: 88/10 to 88/12. The laboratory was set up to begin work. To study the biochemical and molecular factors which regulate triacylglycerol biosynthesis in developing oilseed embryos, a model carrot cell and embryo culture system have been established. Aquobacterium strains which can be used for transformation of the carrot cells have been collected. I have begun the characterization of 5 genomic clones which we have shown to be developmentally regulated during embryogenesis.

Publications: 88/10 to 88/12
No publications reported this period. .

23.021 CRISO089806 MODULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM

ATHERLY A G; PALMER R G; Genetics; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: IOWO2607 Project Type: HATCH Agency ID: CSRS Period: O1 MAR 83 to 30 SEP 87

Objectives: One objective is to test accessions of cultivated soybeans, Glycline max and thewild soybean G. soya, for nitrogen-fixing symbiosis with fast-growing R. japonicum strains and to establish set of differential characteristics to study the genetics of the response. After understanding the genetics of the response soybean lines can be constructed that exclude indigenous R. japonicum strains under field conditions and allow introduction of new more efficient strains. Molecular genetic analysis of nodulation genes in R. japonicum·will be done simultaneously.

Approach: Nodulation and nitrogen fixation will be tested on hydroponically and soil grown plants. Nitrogen fixation rates can be measured by reduction of acetylene to ethylene. Genetic crosses will be conducted by established techniques. Plasmids will be genetically labeled in R. japonicum strains by Tn5 mutagensis and transferred to other strains by conjugation.

Progress: 87/01 to 87/12. Rhizobium japonicum is a slow-growing nitrogen fixing symbiont of soybeans and has great ecnomic

value to agriculture. Recently a fast-growing group of strains were isolated in the People's Republic of China. We have been studying these strains both genetically and with respect to their ability to fix nitrogen on North American soybean cultivars. We have established that all strains have a large plasmid (350-380 megadaltons) which contains the genes necessary for nitrogen fixation and nodulation ability. This large plasmid can be transferred to other bacteria, which successfully nodulate soybeans, but will not fix nitrogen. Apparently other genes on the chromosome are necessary for effective nitrogen fixation. This plasmid has been mapped with respect to the nitrogen fixation genes and the nodulation genes. In contrast to all other Rhizobium strains, Rhizobium fredii has the genes scattered around the plasmid. We have constructed a restriction endonuclease map of the symbiotic plasmid. In addition, we have evidence for the presence of a high degree of repeated sequences present on the plasmid. Some of the nodulation genes themselves are repeated twice, but the majority of the repeated sequences are not yet identified. We have been able to identify the essential genes for nodulation on a 5.2 kb HindIII and a 2.8kb EcoRI fragment, When these two fragments are present in a strain that has been cured of its symbiotic plasmid, it will nodulate soybeans.

Publications: 87/01 to 87/12
PRAKASH, R.K. and ATHERLY, A.G. 1986.
Plasmids of Rhizobium and their role in symbiotic nitrogen fixation; a review.
Inter. Review of Cytology. 104:1-24.
ENGWALL, K.S. and ATHERLY, A.G. 1986.
Construction of R-prime plasmids and identificatin of the symbiotic genes of Rhizobium fredii. Plant. MO1. Biol. 6:41-51.

DUTEAU, N.M., ATHERLY, A.G. and PALMER, R.G. 1986. Fast-growing Rhizobium japonicum strains are poor nitrogen fixing symbionts of soybeans. Crop Sci. 29:884-889.

PRAKASH, R.K. and ATHERLY, A.G. 1984. Reiteration of genes involved in symbiotic nitrogen fixatin of fast-growing Rhizobium japonicum. J. Bacteriol. 160:785-787.

MASTERSON, R.V. and ATHERLY, A.G. 1986. The presences of repeated DNA sequences and a partial restriction map of the pSym of Rhizobium fredii. Plasmid 16:37-44.

RAMAKIRSHNAN, N., PRAKASH, R.K. and ATHERLY, A.G. 1986. Conservation of IS66 homologue Ti plasmid DNA in Rhizobium fredii plasmid DNA. Plant MO1. Biol. 7:177-188.

MASTERSON, R.V., PRAKASH, R.K. and ATHERLY, A.G. 1985. Sequence conservation of DNA in Rhizobium japonicum. J. Bacteriol. 163:21-27.

23.022 CRISO099917 MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA

GENDEL S M; Genetics; Iowa State University, Ames, IOWA 50011.

Proj. No.: IOW02805 Project Type: STATE

Objectives: To use molecular genetic techniques to identify, isolate, and characterize the genes which control nitrogen metabolism in Rhiozbia and Bradyrhizobia. This will make it possible to understand how nitrogen fixation is controlled in these species, and to eventually use this information in constructing more efficient strains for inoculating soybeans.

Approach: Mutants in nitrogen metabolism will be isolated from strains of Rhizobia and Bradyrhizobia using several different techniques. These mutants will then be used to identify and isolate DNA fragments which carry the affected genes. The isolated genes, and adjacent control regions, will be sequenced to identify potential regulator binding sites. These genes will also be used to construct gene fusions which will allow the mechanisms which regulate their expression to be studied in detail.

Progress: 88/01 to 88/12. The most important aspect of nitrogen metabolism in Rhizobia is the ability to fix nitrogen in legume nodules. The nitrogen-fixing cells must adapt metabolically to the anaerobic conditions necessary for nitrogenase activity. My research has focused on understanding the genetic mechanisms necessary for cells to make this adaptation to anaerobic conditions. Because this work requires the development of techniques for screening of oxygen regulated gene clones under various environmental conditions, I have initially focused on developing an E. coli based model system for the isolation of oxygen regulated bacterial promoters. This system uses shot-gun cloning of chromosomal DNA into a promoter-probe plasmid, followed by screening of the resulting clones for oxygen regulated promoter activity. Using this technique we have been able to isolate at least 10 oxygen regulated promoters, and have begun detailed characterization of their activity. Approximately half of the promoters express only under anaerobic conditions, half express only under aerobic conditions. We are in the process of fine-scale mapping of these promoters, and will be subcloning and sequencing the minimal regulatory regions from each.

Publications: 88/01 to 88/12 IMSANDE, J. and GENDEL, S. Nitrogen fixation and metabolic regulation of the gas diffusion barrier in legume nodules. In submission, Physiologia Plantarum.

23.023 CRISO144731 MOLECULAR ANALYSIS OF A SOYBEAN TRANSPOSABLE ELEMENT

SCHNABLE P S; PALMER R G; Genetics; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: 3625-21000-001-03S
Project Type: COOPERATIVE AGREE.

Agency ID: ARS Period: O1 FEB 89 to 31 JAN 90

Objectives: To clone and sequence the transposable element present at the w4 locus in the w4-mutable soybean line.

Approach: DNA will be isolated from soybean plants carrying the w4-mutable allele and their wild-type revertant siblings. The DNA will be used for two purposes. First, Southern analyses will be used to determine the step in the anthocyanin biosynthetic pathway encoded by the w4 locus. Heterologous probes for genes in anythocyanin biosynthesis have been obtained from a number of sources. The gene containing the transposable element will exhibit different restriction patterns in the mutable and revertant siblings. A second use for the soybean DNA will be the construction of genomic libraries. Recombinant phage containing w4 gene sequences will be isolated from genomic libraries of mutable and revertant plants. Restriction maps of the w4 locus will indicate the site of insertion of the element. The insert and the bordering sequenced of the w4 locus will be sequenced by using the Maxim-Gilbert techniq.

23.024* CRISO004941 CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS

POLLAK E; Statistics; Iowa State University, Ames, IOWA 50011.

Proj. No.: IOW01448 Project Type: STATE Agency ID: SAES Period: 01 JUL 59 to 01 JAN 99

Objectives: Study of population genetics, with particular reference to balanced polymorphisms maintained by natural selection occurring in human and other species. Consultation on mathematical problems arising from workers in genetics.

Approach: Procedure will consist partly of the examination of theoretical models and will be partly in cooperation with individuals who have collected or are collecting data on genetic populations.

Progress: 88/01 to 88/12. E. Pollak provided assistance to Dr. A. R. Hallauer of the Department of Agronomy, who asked a question concerning covariances between relatives when a population originally has a Hardy-Weinberg structure and successive generations are produced by self fertilization. Let FS(subscript n) be the mean of a full sib family resulting from a cross between two plants that are produced after n generations of selfing. It was verified that the covariance between FS(subscript 0) and FS(subscript 4) is equal to the covariance between full sib offspring of individuals of generation O. Assistance was also provided to Mr. Brad Hedges, a student in the Department of Agronomy. He was faced with the problem of calculating what family size is large enough so that, if there are two possible sets of underlying frequencies of K types of offspring of a cross, the probabilities of the two kinds of misclassification are each 0.025. Professor C. P. Cox of the Department of Statistics and E. Pollak collaborated in solving this problem. Previously, the solution was known only if there are two types of offspring.

Publications: 88/01 to 88/12

JUNG, Y. C., ROTHSCHILD, M. F., FLANAGAN, M. P., POLLAK, E. and WARNER, C. M. Genetic variability between two breeds based on restriction fragment length polymorphisms (RFLPs) of major histocompatability complex class I genes in the pig.

23.025 CRISO131156 EFFICIENCY OF NITROGEN FIXATION

DAVIS L C; Biochemistry; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KANO0648 Project Type: STATE
Agency ID: SAES Period: O1 JUL 87 to 30 JUN 90

Objectives: This research has two different specific objectives. First, mutagenesis and DNA sequencing will be used to determine amino acid substitutions that alter the function of the Fe protein of nitrogenase from Klebsiella pneumoniae. Mutations will be produced ex situ (in E. coli) and transferred back into K.pneumoniae to characterize the functional nature of the lesions induced. DNA sequencing will be used to identify the locations within the protein sequence that have been mutated.

Approach: Second, 2 dimensional gel electrophoresis and isoelectric focussing will be used to identify proteins specific to root nodules in Glycine max (soybean cultiva BAY) and G. tomentella, and in an interspecific hybrid. Comparison of the proteins produced during nodulation rhizobial strains specific to each of the parental species will reveal whether there are common switching mechanisms in the hybrid that respond to the infection process per se or whether there are species specific switches that turn on only genes associated with a particular genome in the infection process.

Progress: 88/01 to 88/12. This research has been focused on efforts to understand the way in which the host plant genome determines the infection process for different strains of rhizobium. Work has been continued on the way in which hybrids of Glycine $\max x$ Glycine tomentella respond to different strains of rhizobia including both fast and slow growers. Gel electrophoresis of enzymes and proteins specific to root nodules has been expressed. This work was reported at the 7th international symposium on nitrogen fixation, Cologne, Germany. This year a method was developed for distinguishing between acetylene reduction and endogenous ethylene production. It depends on the use of specific infrared absorption bands of the undeuterated and deuterated acetylenes and ethylenes to distinguish between ethylene derived from deuterated acetylene and ethylene produced from other substrated without deuterium. A paper detailing the procedure is in press. A fast-growing wide host range rhizobium isolated from a prairie legume Amorpha has been further characterized for its host range. This is the first report of isolation of such an organism with the ability to nodulate soybean in North America. Its relationship to other fast-growing rhizobia isolated in China is being determined by DNA

restriction mapping.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

23.026 CRISO097318 GLYPHOSATE DEGRADATION AND METABOLISM IN MICROORGANISMS

BRAYMER H D; Microbiology; Louisiana State University, Baton Rouge, LOUISIANA 70803. Proj. No.: LABO2483 Project Type: HATCH Agency ID: CSRS Period: O1 JAN 86 to 31 DEC 90

Objectives: To determine the pathway of glyphosate degradation in the Pseudomonas-like microorganism PG2982. Characterize and modify the transport system used by PG2982 to take up glyphosate. To clone the genes involved in glyphosate resistance (AroA) and glyphosate catabolism into E. coli and then into various plants including the soybean.

Approach: The degradation pathway will be determined by identifying labeled products produced from C labeled glyphosate by PG2982 and by using mutants of the organism that are unable to metabolize the herbicide. The transport system will be studied using mutants and purification of the transport proteins. Cloning of the genes will utilize plasmid and phage vectors and an auxotrophic E. coli recipient. The DNA from the E. coli clones will then be subcloned into appropriate plant vectors. These will then be used to introduce the genes into various plants.

Progress: 88/01 to 88/12. Pseudomonas sp. strain PG2982 is highly resistant to the herbicide glyphosate; a potent inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase, the product of the aroA gene of E. coli. We have isolated a plasmid carrying a 2.4-kilobase pair (kb) fragment of DNA from PG2982 capable of increasing the glyphosate resistance of E. coli cells. Preliminary subcloning data suggested that the fragment did not contain the entire gene for glyphosate resistance. In order to isolate a larger fragment of DNA containing the entire gene, a library of large DNA fragments from PG2982 was constructed using a bacteriophage vector. The originally isolated DNA was used to probe this library and a lambda clone carrying the entire gene from PG 2982 was isolated. Subcloning of a 2kb DNA fragment carrying this gene has again resulted in a plasmid capable of increasing glyphosate resistance in E. coli. A protein with a molecular weight of approximately 40,000 is encoded by this plasmid. It is not able to complement the aroA mutation of E. coli strain LC3 and will not hybridize to the e. coli aroA gene. Also, glyphosate cannot be broken down by E. coli cells containing the plasmid. The nucleotide sequence of the gene has revealed the presence of an open reading frame able to encode a protein with a calculated molecular weight of 39,396. Computer analysis has not revealed any significant similarity to other known genes.

Publications: 88/01 to 88/12
FITZGIBBON, J. and BRAYMER, H.D. 1988.
 Phosphate starvation induces uptake of
 glyphosate by Pseudomonas sp. strain
 PG2982. Appl. Environ. Microbiol.
 54:1886-1888.

ROSS, T.K., ACHBERGER, E.C. and BRAYMER, H.D. 1987. Characterization of the Escherichia coli modified cytosine restriction (mcrB) gene. Gene. 61:277-289.

ROSS, T.K., ACHBERGER, E.C. and BRAYMER, H.D. 1989. Identification of a second polypeptide required for McrB restriction of 5-methyl cytosine-containing DNA in Escherichia coli K-12. Molecular and General Genetics. In press.

MURPHY, K.E. and BRAYMER, H.D. 1988.

Molecular cloning and characterization of a genetic region from Serratia marcescens involved in DNA repair. Molecular Microbiology. In press.

23.027 CRISO095653 ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIDE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM

GAYDA R C; Microbiology; Louisiana State
University, Baton Rouge, **LOUISIANA** 70803.
Proj. No.: LABO2450 Project Type: HATCH
Agency ID: CSRS Period: O1 MAY 85 to 30 SEP 89

Objectives: Isolate genes involved in exopolysaccharide biosynthesis in Rhizobium japonicum. Into isolated genes construct transcriptional fusions to lacZ (B-galactosidase). Reintroduce the transcription fusions into R. japonicum and investigate gene expression under different physiological conditions. Localize the genes in the genetic map of R. japonicum. Isolate neighboring R. japonicum DNA regions.

Approach: The R. japonicum genes for exopolysaccharide biosynthesis-carbohydrate synthesis will be selected using E. coli capsular polysaccharide mutants and a recombinant DNA gene library of R. japonicum. Transcriptional fusions will be isolated in E. coli using the opposite selection of loss of enzyme function and restoration of original E. coli mutant phenotype. Mutated clones will be mobilized by an R transfer plasmid and reintroduced into R. japonicum strains. Concurrently, exopolysaccharide mutants of R. japonicum will be isolated by transposon (tn5) mutagenesis. Isolated genes will be tested for complementation in these R. japonicum transposon mutants. Neighboring DNA regions will be isolated using the cloned genes as probes, and will be tested for symbiosis functions.

Progress: 88/01 to 88/12. Production of exopolysaccharide (EXO) by rhizobia has been linked with efficient bacterial invasion of plant roots during nodulation. EXO-deficient mutants of Rhizobium fredii USDA191 were isolated by Tn5 insertion mutagenesis. Five phenotypically unique EXO deficient mutants were investigated for EXO synthesis and nodulation ability. The EXO synthesized by

these mutants was analyzed for polysaccharide composition by column chromatography and thin layer chromatography. Three mutants still synthesized neutral glycan polysaccharide at various levels compared with wild type. Two mutants were deficient in both the neutral glycan and capsular polysaccharide (CPS), but made effective nodules on Glycine soja cv. Peking, although with decreased efficiency. On a different Glycine soja cv. Maturation Group II, one mutant had lack of nodulation ability; it grew poorly compared with wild type bacteria and its EXO deficiency was complemented by a cloned R. meliloti exoC loci. Our data implies that exopolysaccharide including neutral glycan is not essential for R. fredii to nodulate soybeans. Thus the role of exopolysaccharide during infection is different for R. fredii than that observed with R. meliloti. The laboratory is in the process of isolating EXO genes from a gene library of R. fredii chromosomal ONA that complement the Tn5 mutants.

Publications: 88/01 to 88/12

KO, Y.H. and GAYOA, R. 1988. Extracellular neutral polysaccharide production in sufficient for nodulation of Glycine soja by Rhizobium fredii USOA191. 1988 Abstracts of Annual Meeting of American Society for Microbiology.

KO, Y.H. and GAYDA, R.C. Exopolysaccharide including neutral glycan is not essential for nodulation of Glycine soja by Rhizobium fredii USOA191. To be submitted to Journal Applied and Environmental Microbiology.

23.028 CRISO098434 PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH DNA METHYLATION

EHRLICH K C; EHRLICH M; Southern Regional Res Center; Po Box 19687, New Orleans, LOUISIANA 70179.

Proj. No.: LABR-8503101 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 85 to 30 SEP 88

Objectives: Proj. 8503101. Upgrade soybean meal for human and animal consumption by reducing undesirable components and increasing desired components. Use biotechnological approaches to determine how methylation of ONA in plants controls gene expression.

Approach: Plant proteins involved in specifically recognizing methylated ONA sequences and plant enzymes responsible for ONA methylation will be isolated. Such proteins from plant sources have not yet been examined. Making such proteins available will allow us to analyze the effect of gene structure on plant gene function and to modify the structure of cloned plant genes in vitro. Such modification could modulate their expression for the purposes of genetic engineering. Specifically, two enzymes will be isolated: a ONA methyltransferase, which methylates specific sites in the plant genome, and methylated ONA binding protein(s) (MOBP).

Progress: 87/01 to 87/12. The presence of a sequence-specific ONA binding protein which prefers methylated cytosine over cytosine in the binding site has been determined for the first time in crude nuclear extracts from pea, soybean, and cauliflower. The 35 base-pair oligonucleotide, TMG CMG AGG MGG CAT AAA TMG CMG TGA MGA TCA GC AGM GGM TCC GMC GTA TTT AGM GGM ACT GMT AGT CG prepared synthetically, was retained on a 10% polyacrylamide gel when electrophoresis was performed in the presence of crude nuclear extracts. Whereas the homologous methylated oligonucleotide competed for binding, the unmethylated oligonucleotide analogue, other non-homologous oligonucleotides of the same length, a digest of M. luteus ONA, and poly dI.poly dC failed to compete, even when used in a 50-fold molar excess. A fully methylated ONA, XP12, however, competed well for binding. These data provide strong evidence for the presence of a plant MOBP, analogous to that isolated from mammalian tissues. Such proteins may play a role in the negative regulation of gene expression during tissue development.

Publications: 87/01 to 87/12 EHRLICH, K. and MALBROUE, C. 1987. Isolation of DNA methyltransferase from Plants. Fed. Proceedings. 46: 2257 (Abst. 1928).

23.029 CRISO142988 DETERMINATION OF RELATEDNESS OF SELECTED LINKAGES IN SOYBEAN GENES

OEVINE T E; Beltsville Agr Res Center, Beltsville, MARYLAND 20705. Proj. No.: 1275-22000-007-00D

Project Type: INHOUSE

Agency ID: ARS Period: 19 APR 88 to 18 OCT 88

Objectives: Establish chromosomal location of specific genes in soybean.

Approach: Genes selected from the genetic type collection suitable for linkage studies will be used on the basis of clear phenotypic expression of traits during early seedling ontogeny. Electrophoretic isozyme analysis will be correlated with expression. Map units will be determined using electrophoretic isozyme analysis. This work will be dovetailed with restriction fragment length polymorphism of Schaff and Matthews at the samelaboratory.

Progress: 88/01 to 88/12. Thirty-four pairs of genes were tested for genetic linkage using F2 analysis by the product method. Combinations tested wre e and f, e and rjl, e and t, e and yl7, f and rjl, f and t, f and yl7, f and yl2, frl and rj4, fr2 and pl, fr2 and rj2, fr2 and rj4, fr2 and w1, p1 and rj2, p1 and w1, p1 and y10, p1 and y17, p2 and rj1, p2 and y17, pc and y9, pc and y13, rjl and t, rjl and y 17, rjl and yl2, rj2 and yl0, rj2 and yl3, rj2 and yl7, rj4 and w1, rj4 and y12, rj4 and y17, t and y17, t and y12, and w1 and y17. All combinations tested were governed by independent assortment except e and t, f and rjl, and t and yl2. In cooperation with researchers at the University of New Hampshire we tested eight electrophoretic loci (Aco2,

Adhl, Dial, Got, Idhl, Mpi, Pgdl, Pgml) and three morphological loci (Rj2, Wl and T) for recombination frequency in the F2 generation. All gene combinations were found to assort independently except Adhl and Wl. This knowledge is a significant fund of new information useful in constructing a genetic map of soybean that will delineate which genes are linked together on the chromosome and which are inherited independently. Knowledge of the location of genes on the chromosome is of great value to plant breeders, both in the commercial and public sector, in estimating the population sizes needed in progeny populations from hybridization in order to recover agronomically desirable recombinant individuals with the best qualities of both parent lines.

Publications: 88/01 to 88/12

DEVINE, T.E. 1988. Role of the nodulation restrictive allel e Rj4 in soybean evolution. J. Plant Physiol. 132:453-45 5.

DEVINE, T.E., KUYKENDALL, L.D. and O'NEILL, J.J. 1988. DN A homology group and the identity of Bradyrhizobial strains producing rhizobitoxine-induced foliar chlorosis on soybean. Crop Sci. 28:939-941. KUYKENDALL, L.D., ROY, M.A., O'NEILL, J.J. and DEVINE, T.E. 1988. Fatty acids, a ntibiotic resistance, and deoxyribonucl eic acid homology groups of Brady- rhizobium japonicum. Interntl. Jour. Syst. Bact. 38:358-361.

23.030* CRISO141190 ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS

SCHAEFFER G W; Beltsville Agr Res Center, Beltsville, MARYLAND 20705. Proj. No.: 1275-22000-004-00D

Project Type: INHOUSE Agency ID: ARS Period: O1 APR 86 to 31 MAR 91

Objectives: Identify, isolate, clone and characterize genes fine structure and functionassociated with elevated lysine levels for plants regenerated from cells resistant to aminoethylcysteine & cells insensitive to inhibitory levels oflysine plus threonine. The first enzyme to be studied in detail is diaminopimelate dehydrogenase whose presence has been demonstrated in rice.

Approach: The structure and function of the gene(s) along the lysine pathway in cereals will be characterized by: a) profiling electrophoretically the seed storage proteins of mutants & the amino acid composition of 30-50 proteins established; b) polyadenylated RNA of variants will be translated in vitro & early polypeptide products separated two dimensionally to estab-lish direct relationships between phenotypes & protein composition, cDNA clones & probes for those clones will be created from amino acid sequences & other methods for the study of gene regulation & temporal expression; c) the fine structure & functional promoters of genes will be defined & sequenced. Additionally work will be extended to determine whether insta-bility

induced by tissue culture may be due to the mobilization of DNA elements and to improve plant regeneration from callus and cell suspensions. Beltsville, MD; Rm 127, Bg.O11A; BL-2; 12/85; Scientists & technicians: G. Schaeffer; L.Wenko; F.Sharpe, Jr.; J.Dudley; L.Weaver; L.Baustiloos.

Progress: 88/01 to 88/12. The research has focused on the continued amino acid analyses of single seeds to identify heritable high lysine lines from selfed lines, crosses with original parents and backcrosses to high lysine mutants. Fifth generation seeds are now available and are being prepared for field tests. Tissue culture cell lines of high lysine plants have been established and are being utilized for biochemical isolation of specific proteins and characterization of the lines. Proteins of rice endosperm mutants have been fractionated into solubility classes and the amino acid characteristics determined. The major increase in lysine occurs in the salt soluble globulin fraction. Not only is there a shift in the quantity of individual proteins in the mutant but some types of protein appear to be specifically modified as well in the mutant. Currently unique proteins are being isolated, monitored with 3H-lysine, and will be purified in the weeks ahead. Specific genes will be isolated from these mutant lines. This research will lead to new basic information on the synthesis of lysine in rice and the release of new rice germplasm.

Publications: 88/01 to 88/12

- CHOWDHURY, M.K.U., SCHAEFFER, G.W., SMITH, R.L. and MATTHEWS, B.F. 1988. Mole- c ular analysis of organelle DNA of different subspecies of rice and the genomic stability of mtDNA in tissue cultures of rice. Theor. Appl. Genet.76:533-539.
- SESEK, S., BOROJEVIC, K. and SCHAEFFER, G.W. 1988. In vitro production of dihpaloids via anther culture in wheat. 7th Internatl. Wheat Genetics Sym-posium, Cambridge University, England, July 13-19, 1988. (Abstract).
- SCHAEFFER, G.W. 1988. Segregation for endosperm lysine and protein as well as infertility from crosses of in vitro selected rice. J. Cellular Biochemistry, Proc. 17th Ann. Mtg. UCLA Symp. on Mol. & Cell. Biol., p. 203. (Abstract).

 SCHAEFFER, G. 1988. Segregation for endosperm
- SCHAEFFER, G. 1988. Segregation for endosperm lysine and p rotein as well as infertility from crosses of in vitro selected rice. Proceedings of 22nd Rice Technical Working Group, U. of California, Davis, June 1988. (Abstract).
- SCHAEFFER, G.W. 1988. Segregation for increased lysine from crosses of in vitro selected mutants of rice. Proceedings o f 6th Congress of Federation of Euro- pean Societies of Plant Physiology, Split, Yugoslavia, Sept. 1988. (Abstract).
- SCHAEFFER, G.W. 1988. Role of microspores and anther culture in advancing technologies. In: Advances in Cell Culture, edited by K. Maramorosch and G. H.
- Sato. (Book chapter). Academic Press, N.Y.

23.031* CRISO141166
MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION
OF ORGANELLE AND NUCLEAR GENES

MATTHEWS B F; Agricultural Research Service; Beltsville Agr Res Center, Beltsville, MARYLAND 20705

Proj. No.: 1275-22000-003-00D

Project Type: INHOUSE

Agency ID: ARS Period: O1 APR 86 to 25 JUN 87

Objectives: Develop technologies for bypassing traditional sexual breeding barriers andmaternal inheritance to create variation and unique gene combinations for improving productivity of economically important crop plants, such as wheat, rice and soybean and to map and study the regulation of important genes in these crop systems.

Approach: 1) Transfer portions of chloroplast, mitochondrial and nuclear genomes between species, 2) develop a map and determine the structural organization of mitochondrial genome in the recipient parent and hybrid cell lines, 3) determine the effects of chloroplast transfer on nuclear-chloroplast interaction by monitoring production & regulation of key enzymes (aspartokinase, homoserine dehydrogenases and dihydrodipicolinic acid synthase) involved in synthesis of essential and nutritionally important amino acids, lysine, threonine and methionine from aspartate, and 4) clone nuclear genes encoding these enzymes. Enzyme activities, located mainly inthe chloroplast, will be measured and characterized. Thus, these enzymes will be examined at the gene, mRNA and protein levels to understand mecha- nisms regulating this typical plant biosynthetic pathway. BELTSVILLE, MD; BG 010, RM 9 & 10; BL-1; 12/05/85. B. Matthew, C. Cohen, L. DeBonte.

Progress: 87/01 to 87/06. The cox II gene from the mitochondrion of carrot has been isolated and mapped. A complex intron is present. Portions of the cox II gene and intron have been sequenced to confirm these observations. Research benefited other scientists conducting research in genetic engineering.

Publications: 87/01 to 87/06
NO PUBLICATIONS REPORTED THIS PERIOD.

23.032* CRISO049444 GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS

SCHAEFFER G W; CRISS; MATHEWS; Agricultural Research Service; Beltsville Agr Res Center, Beltsville, MARYLAND 20705.

Proj. No.: 1209-20173-003-00D

Project Type: INHOUSE Agency ID: ARS Period: 01 OCT 84 to 12 AUG 86

Objectives: Terminate 1209-20173-002, accession 0043396. Start 1209-20173-003 with \$300,000 net to bench funds planned for 1209-20173-002 and \$50,000 from \$4.1 million administrative reduction funds. G. Still is NPS contact. Table 1 - 11F.

Approach: Twelve on-going projects will receive additional funding to accelerate research on genetic engineering, gene mapping and transfer, hormonal regulation, membrane structure, and other biotechnologies for improved crop productivity, including control of insects, diseases, and other pests. This high-technology research will improve the fundamental understanding of important agricultural problems and lead to innovative solutions to agricultural problems.

Progress: 84/01 to 84/12. The 160-kilobase chloroplast (ct) DNA of Daucus carota was cloned, and a physical map was constructed. The circular ctDNA map delineates the positions of an inverted repeated region and genes encoding ribosomal RNAs, the large subunit of ribulose biphosphate carboxylase and the 32-kilodalton protein. The ctDNA of a wild species, D. pusillus was also mapped. Although it did show a great deal of variation in restriction endonuclease digestion patterns, the gene positions were found to be conserved. The D. carota 450-kilobase mitochondrial genome was cloned in a bacteriophage lambda vector, and portions of this gene library were partially mapped to the complete circular genome. The ribosomal RNAs and the gene encoding the protontranslocating subunit of the mitochondrial ATPase were localized and mapped. Analysis of flanking regions reveals that these genes are found in only one copy per mitochondrial genome. Changes in gene expression in develoing somatic embryos of D. carota were detected by two-dimensional gel electrophoresis of nascent proteins. Some changes were observed as early as one day following induction of the in vitro developmental sequence by removal of auxin from the culture medium. Putative mutants of D. carota, temperature-sensitive for somatic embryo development, were isolated by a filtration enrichment protocol.

Publications: 84/01 to 84/12
DE BONTE, L.R., MATTHEWS, B.F., and WILSON, K.G. 1984. Variation in plastid and mitochondrial DNAs in the genus Daucus. Amer. J. Bot. 7:932-940.

MATTHEWS, B.F., DE BONTE, L.R. 1985. Chloroplast and mitochondrial DNAs of the carrot and its wild relatives. Plant Molec. Biol. Reporter. (In press).

MATTHEWS, B.F. and WIDHOLM, J.M. 1985.
Organelle DNA compositions and isoenzyme expression in an interspecific hybrid of Daucus. Molec. Gen. Genet. (In press).

DIENER, T.O., OWENS, R.A., and CRESS, D.E. 1984. Plant viroids: new diagnostic methods...agriculture. In: Control of Virus Diseases, E. Kurstak and R.G.

Marusyk, eds., Dekker, New York, pp. 345-360. OWENS, R.A., KIEFER, M.C., and CRESS, D.E. 1985. Biological activity of cloned ...cDNAs. In: Subviral Pathogens of Plants and Animals, Maramorosch and McKelvey, eds., Academic Press, NY. (In press).

HAMMOND, R.W., KIEFER, M.C., CRESS, D.E. and OWENS, R.A. 1984. Probing viroid structure-function...cDNAs. In: Molec. Form and Function of Plant Genome, Plenum, NY. (In press).

23.033 CRISO049908 GENE EXPRESSION IN PLANT DEVELOPMENT

VACANT; RHDDES R R; FRANK R F; Plant Molecular Genetics Lab Genetica & Germplsm Inst; Beltsville Agr Res Center, Beltsville, MARYLAND 20705.

Proj. No.: 1275-22410-001-00D

Project Type: INHDUSE

Agency ID: ARS Period: O1 APR 85 to O1 APR 88

Objectives: To (1) examine developmental regulation of gene activity in higher plants using gene transfer methods, and (2) develop methods for tagging or isolating genes in soybeans using transposable elements.

Approach: The developmentally regulated soybean seed lectin gene will be placed in a series of Agrobacterium tumefaciens Ti-plasmid vectors and moved into tobacco cells and plants. Various tissues of transformed tobacco plants which contain soybean sequences in the genomic DNA will be assayed for expression of lectin messenger RNA and protein. Soybean lines or related Glycine species which normally do not express lectin will be transformed and regeneration of whole plants will be attempted. In the second objec- tive, sequences related to a lectin insertion element will be examined and experiments performed to determine whether there is a correlation of these sequences to other soybean phenotypes or mutable traits. Experiments will be initiated to induce mobility of the lectin element or other potentially mobile sequences within the soybean genome. Approaches include investiga- tion of variation caused by radiation or other mutagenic agents and varia- tions induced by the process of tissue culture.

Progress: 88/01 to 88/12. Two genes which encode key enzymes in the synthesis of the dark purple pigments found in flowers and seeds have been isolated from soybean. More than one copy of each of these genes was found in the genetic material of soybean. One gene, called chalcone synthase (CHS), exists in about 4 to 7 copies, and the other gene, designated phenylalenine ammonia lyase (PAL) has 2 to 3 copies in soybean. The purpose was to obtain molecular probes to study the organization of these genes in soybean and for examination of novel regulatory mechanisms present in the anthocyanin biosynthesis pathway of this system. The genes were identified by sequence homology to the CHS gene of Antirrhinum majus and the PAL gene of P. vulgaris. The representative CHS gene that was characterized contains at least one intron at the same relative position as intron I of A. majus and no intron corresponding to intron II of A. majus. These clones will be valuable as molecular probes to study the regulation of genes in soybean.

Publications: 88/01 to 88/12

RHDDES, P.R. and VDDKIN, L.D. 1988.

Drganization of the Tgm family of transposab le elements in soybean. Genetics 120: 597-604.

CHANDLEE, J.M. and VDDKIN, L.D. 1989. Unstable expression of a soybean gene durin g seed coat development. Theor. Appl. Genetics. Accepted December 1988.

23.034 CRISO049513 GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS

MATHEWS B F; Seed Research Lab Plant Genetics & Germplsm Inst; Beltsville Agr Res Center, Beltsville, MARYLAND 20705.
Proj. No.: 1275-21000-009-00D

Project Type: INHDUSE Agency ID: ARS Period: O1 MAR 85 to O1 MAR 90

Objectives: To construct a chromosomal linkage map in soybean using restriction frag-ment length polymorphisms with DNA markers.

Approach: DNA will be extracted from leaf tissue of two parental soybean lines and examined on Southern blots for polymorphisms using a number of restriction enzymes. A genomic library of soybean will be screened to isolate random clones which represent single or low copy sequences. These will be used asprobes in the Southern blotting experiments to determine whether they can serve as markers to distinguish between the two parental varieties. Dnce acollection of markers has been obtained, the markers will be mapped with respect to one another and to other morphological plant traits by examiningtheir segregation patterns in backcrossed plant material. Additionally, a somatic approach to mapping may be undertaken by examining the behavior of markers during chromosome loss induced in tissue culture.

Progress: 88/01 to 88/12. We have constructed the following genomic and cDNA libraries from soybean: 1) a lambda gtll cDNA expression library from mRNA of developing soybean seedlings; 2) a lambda ZAP library from mRNA of soybean cotyledons; 3) a genomic DNA library from a PstI digestion. From these libraries we have identified 24 clones which can detect polymorphisms between at least two of five parents on Southern blots. The data have been cataloged. Recently, nine probes have been sent to Gordon Lark at the University of Utah for F2 mapping. We are screening Noir and Minsoy as parents and have also added three other parents and two crosses to link certain phenotypes with our RFLP map. This may lead to the cloning of genes involved in nodulation and genes involved in resistance to phytophthora. The RFLP map will be of use to commercial soybean breeders by providing chromosome markers and linkages.

Publications: 88/01 to 88/12

SCHAFF, D.A., BAUCHAN, G.R. and MATTHEWS, B.F. 1988. Refine-ment of in situ hybr idization for alfalfa chromosome identification. Genome 30:32. (Abstract).

BAUCHAN, G.R., SCHAFF, D.A. and MATTHEWS, B.F. 1988. Developments towards the production of a chromosomal gene map of alfalfa. Proceedings of 31st Nort h American Alfalfa Improvement Conference. (Abstract).

BAUCHAN, G.R., SCHAFF, D.A. and MATTHEWS, B.F. 1988. Utili- zation of DNA probes for identifying alfalfa chromosomes. Mid-Atlantic Plant Molecular Biology Soc iety, p. 30. (Abstract).

BAUCHAN, G.R., SCHAFF, D.A. and MATTHEWS,
B.F. 1988. Location of specific genes on
alfalfa chromosomes. Crop Sci. (Abstract).

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identifying alfalfa chromosomes.
Mid-Atlantic Plant Molecular Biology Soc
iety, p. 30. (Abstract).

23.035 CRISO034144 STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE

VODKIN L O; USDA-ARS-northeastern Region; Seed Research Laboratory, Beltsville, MARYLAND 20705.

Proj. No.: MD-1108-20583-007 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 83 to 31 AUG 87

Objectives: Project 8300593. To determine the structure of a 3.4 kb insertion element which interrupts a soybean lecin gene and to examine the nature of sequences related to this element which are present elsewhere in the soybean genome. The lectin gene insertion has certain features analogous to transposable elements.

Approach: The complete nucleotide sequence of the lectin gene insertion element will be determined using Bal-31 nuclease to create an overlapping set of deletion fragments which ae subcloned into the single-stranded M13 phage vectors and sequenced by the dideoxy method. Synthetic primers will be used to overlap certain regions if necessary. Potential open reading frame regions within the insertion element will be cloned into experession vectors. Genomic regions harboring sequences related to the lectin insertion element will be isolated from soybean libraries and characterized by restriction mapping and sequencing where appropriate.

Progress: 83/09 to 87/06. The recessive allele for soybean seed lectin results from the insertion of a DNA segment (designated Tgml) into the coing region of the gene. The termini of the 3550 bp element display features characteristic of a transposable element and have sequence similarity to the Spm element of maize and the Taml element of snapdragon. The complete sequence of the element revealed a complex and highly structured border at its 5' and 3' ends which consist of repeat units having dyad symmetries. In order to characterize related members of this family, a lamb library was screened for clones having homology to Tgml. Six isolates were characterized by restriction mapping and partial sequencing. Restriction mapping revealed a diverse internal organization for the elements but comparable regions between the elements could be discerned. The elements vary in size from 1.6 kb to greater than 12 kb. Synthetic oligonucleotides made to the 13 bp termini of Tgml were used to locate the 5' and 3' ends of the related elements by priming sequencing reactions. Except for occasional single base substitutions and small deletions. the 13 bp termini and repeat border regions are highly similar in sequence to those of Tgml.

Most of the related elements have additional copies of the repeating unit motif at the 3' ends as compared to Tgml. Only one of the smaller elements contained both 5' and 3' termini and is apparently a deletion derivative.

Publications: 83/09 to 87/06

RHODES, P.R. and VODKIN, L.O. (1985) Highly structured sequence homology between an insertion element and the gene in which it resides. Proc. Natl. Acad. Sci. USA 82: 493-497.

VODKIN, L.O., RHODES, R.R., CHANDLEE, J.M. and HARDING, R.H. (1986) Lectin genes and a transposable element in soybean. In "Biotechnology for Solving Agricultural Problems: Beltsville Symposia in Agricultural Research, vol. 10, pp.99-113.

VODKIN, L.O. and RHODES, P.R. (1986) Common structural patterns between the soybean lecti insertion and transposable elements in other plant species. In: Molecular Biology of Seed Proteins and Lectins.

23.036 CRISOO49891 MOBILE ELEMENTS IN THE SOYBEAN GENOME

VODKIN L O; WU M; Agricultural Research Service; University of Maryland, Catonsville, MARYLAND 21227.

Proj. No.: 1275-22410-001-01S

elements.

Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 15 MAR 85 to 30 JUN 88

Objectives: To examine for the presence of mobile genetic elements in the soybean genome in an effort to developmethods to tag or isolate genes in soybeans using transposable

Approach: Sequences related to a soybean insertion element, Tgml, will be examined and blot hybridization experiments will be performed to determine whether there is a correlation of these sequences to other soybean phenotypes or mutable traits. Experiments will be initiated to induce mobility of this element or other potentially mobile sequences within the soybean genome. Approaches include investigation of variation caused by radiation or other mutagenic agents or variation induced by the process of tissue culture.

Progress: 87/01 to 87/12. Three alleles of the R locus of soybean which is responsible for modifying seed coat pigmentation have been identified. We have observed both somatic and germinal instability in the expression and inheritance of rm arising spontaneously from the original rm stock. Somatic (non-heritable) instability is evident when single plants produce mixtures of seed (black + ringed mutable or brown + ringed mutable). Germinal (heritable) alterations at the R locus are characterized by plants which produce all black or all brown seed. While the all brown-seeded plants breed true for their phenotype, the all black may breed true or may segregate 3:1 for black:ringed mutable seed. On occasion, subsequent generations arising from these segrated sublines continue to show instability

at the brown (rr) or black (RR) loci by producing ringed or black + ringed seeds. We looked for evidence of an active Tgm transposable element in these lines and in soybean isolines with other mutations affecting expression of the anthocyanin pathway in flowers and seeds. Southern blots of soybean DNA digested with a variety of restriction enzymes and probed with subcloned regions of Tgm1 revealed banding patterns consistent with transposable element action. The research will benefit molecular geneticists interested in gene expression and inheritance.

Publications: 87/01 to 87/12
CHANDLEE, J.M. and VODKIN, L.O. 1987.
Identification and characterization of active transposable element systems in soybean. In International Symposium on Plant Transposable Elements, Univ. of Wisconsin, Madison. p.50. (Abstr.).
CHANDLEE, J.M. and VODKIN, L.O. 1987.
Analysis of active transposable element systems in soybean. Soybean Genetics Newsletter 14:256-257.

23.037 CRISO131260 REGULATION OF FATTY ACID SYNTHESIS PROTEINS IN SOYBEAN

OHLROGGE J B; Botany & Plant Pathology; Michigan State University, East Lansing, MICHIGAN 48824.

Proj. No.: MICLO 8040 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 86 to 30 SEP 89

Objectives: PROJECT 8603145. In oil seed crops such as soybean, fatty acid synthesis plays two different roles: an essential housekeeping function in the synthesis of membranes in every cell and a specialized developmental function in the production of oil for storage in the seed.

Approach: The objective of this project is to examine the regulation of fatty acid synthesis gene expression for both these functions by examining two types of tissue--rapidly dividing leaf tissue (where fatty acids are used primarily for membrane production) and nondividing seed tissue (where fatty acids are used primarily for oil storage). To achieve the above objective we shall use antibodies and cDNA probes to measure levels of FAS proteins and their mRNA's in both rapidly dividing tissue (housekeeping) and nondividing oil producing tissue (developmental). Our goal is to examine whether the synthesis of FAS proteins when produced for a housekeeping role is regulated transcriptionally and/or post-transcriptionally in a different way than when produced for oil production. This project therefore offers an opportunity to examine how plants regulate genes which are needed for two divergent functions.

Progress: 88/01 to 88/12. At least two forms of acyl carrier protein (ACP) occur in all higher plant species examined and these forms are expressed differently in different tissues. Recently our lab has isolated a genomic clone of ACP from Arabidopsis and found that it

contains three introns; two in the transit peptide and one just downstream of the phophopantetheine attachment site. We have also isolated a cDNA clone for ACP by immunological screening of a spinach root library. The derived amino acid sequence of this clone matches the N-terminal amino acid sequence of the minor form of ACP isolated from spinach leaf. This suggests that the same gene for ACP-II is expressed in both leaves and roots. In order to examine the consequences of overexpression of ACP in vivo, we have transformed tobacco plants with spinach ACP-I. A plasmid was constructed containing a fusion between the tobacco RuBisCo small subunit promoter and transit peptide and mature spinach ACP-I. The recombinant gene is under light regulation and is expected to produce the precursor ACP whose transit peptide would be cleaved as it is transported into the chcloroplast. Western blot analyses of leaf homogenates from ACP-transgenic plants demonstrate that the spinach ACP accumulated to levels in excess of the endogenous tobacco ACPs and that the transit peptide is leaved. Normally apoACP is not detected in plants. However, in the plants transformed with the spinach ACP approximately 50% of the spinach ACP is in the apo form.

Publications: 88/01 to 88/12

HANNAPEL, D.J. and OHLROGGE, J.B. 1988.

Regulation of Acyl Carrier Protein

Messenger RNA Levels during Seed and Leaf

Development. Plant Physiol. 86:1174-1178.

ELHUSSEIN, S.A., MIERNYK, J.A. and OHLROGGE,

J.B. 1988. Plant holo-(acyl carrier

protein) synthase. Biochem. J. 252:39-45.

GUERRA, D.J., DZIEWANOWSKA, K., OHLROGGE,

J.B. and BEREMAND, P.D. 1988. Purification

and Characterization of Recombinant Spinach

Acyl Carrier Protein I Expressed in

Escherichia coli. J. of Biological

Chemistry 263:4386-4391.

MATTOO, A.K., CALLAHAN, F.E., MEHTA, R.A. and

OHLROGGE, J.B. 1989. Rapid In vivo

Acylation of Acyl Carrier Protein with

Exogenous Fatty Acids in Spirodela

23.038 CRISO130667 HYBRID VARIEGATION IN PHASEOLUS VULGARIS

oligorrhiza. Plant Physiol. in press.

SEARS B B; Botany & Plant Pathology; Michigan State University, East Lansing, MICHIGAN 48824.

Proj. No.: MICLO1513 Project Type: HATCH Agency ID: CSRS Period: O1 DEC 86 to 30 NOV 91

Objectives: Many of the F(2) progeny in wide crosses of the common bean Phaseolus vulgaris display clonal patterns of leaf variegation. Previous investigations of leaf variegation would most strongly implicate one of the following mechanisms: biparental inheritance of the plastids could occur with subsequent incompatibility of plastid and nuclear genetic systems; or a controlling element could be activated, affecting the expression of other loci which directly or indirectly contribute to chlorophyll biosynthesis and/or chloroplast differentiation.

Approach: To discriminate between these alternatives, several experiments are being conducted in parallel. Infectivity tests have ruled out the possibility of a viral cause of the F(2) leaf variegation. Chloroplast DNA from the parental and offspring lines is being analyzed to allow an assessment of the extent of biparental transmission of chloroplasts in crosses by the use of restriction fragment polymorphisms and Southern blotting. Standard backcrosses and sib crosses are being performed in order to determine the genetic basis of the variegation phenomenon, including an assessment of the number of loci involved and whether non-Mendelian genes or Mendelian genes are responsible.

Progress: 88/01 to 88/12. Previous genetic studies suggested that plastids were transmitted from both parents at a relatively high frequency in crosses of the common bean Phaseolus vulgaris. A reinvestigation was undertaken to assess the manner of non-Mendelian inheritance in Phaseolus vulgaris using molecular markers. Restriction enzymes and conditions of gel electrophoresis were tested to establish conditions which allow visualization of restriction fragment length polymorphisms between different bean cultivars. One of these polymorphisms was identified using a spinach cytochrome f gene probe. Recriprocal crosses were made between cultivars varying at this locus to produce F(1) progeny. Chloroplast DNAs (cpDNAs) were isolated from parents and F(1) progeny of the common bean. Using a polyacrylamide gel system, cpDNA restriction fragment length polymorphisms were identified in the parental lines. The cpDNA restriction fragments of the F(1) progeny were identical to those of the maternal parents. The Southern hybridization analysis indicated that the F(1) progeny did not possess cpDNA from the male parent at the 1% level of detection. These results show that the transmission of chloroplasts in sexual crosses of Phaseolus vulgaris is primarily by uniparental maternal inheritance.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

23.039 CRISO093360
GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVED AGRONOMIC PROPERTIES

CHELM B K; Microbiology and Public Health; Michigan State University, East Lansing, MICHIGAN 48824.

Proj. No.: MICLO3257 Project Type: STATE Agency ID: SAES Period: 27 JUL 84 to 31 DEC 87

Objectives: This project has two main objectives. The first is the construction by recombinant DNA methods of vector systems capable of transfer and expression of foreign genes in Rhizobium japonicum. In particular, we plan to engineer vectors which allow regulated rather than continuous expression of the inserted genes. This focus on the regulated expression of the inserted gene systems is unique and crucial since certain genes might

impart improved agronomic properties if expressed when the bacteria are located in the soil but could impart deleterious effects on plant yield if expressed in nodules. The second objective is the construction of genetically engineered improved R. japonicum strains with our vector systems.

Approach: These strains will be made by cloning genes from other microbial systems into our expression vectors and then transferring them to standard inoculum strains of R. japonicum. The engineered strains will then be tested for improvement in properties such as increased nitrogen fixation per plant, decreased inhibition of nitrogen fixation by applied nitrogenous compounds, increased resistance to disease of nodulated plants and increased tolerance of plants to herbicides.

Progress: 84/07 to 87/12. In order to construct vectors for regulated gene expression in Bradyrhizobium japonicum a variety of transcription promoters have been characterized from this organism. These include the promoters for three nitrogenase operons (nifDKE, nifH and nifB), two glutamine synthetase operons (glnA and glnII) and a heme biosynthetic operon (hemA). In addition several other transcription promoters have been localized but not sequenced including a ribosomal RNA operon, an operon essential for formate utilization, several operons that are expressed at high levels in bacteroids and several operons that are repressed in bacteroids. In total this represents examples of the following classes of promoter signals: 1) promoters expressed at high levels in bacteroids only, 2) promoters expressed at high levels in vegetative cells only, 3) promoters expressed at high levels in both vegetative cells and bacteroids, and 4) promoters expressed at low levels in both vegetative cells and bacteroids, and 4) promoters expressed at low levels in both vegetative cells and bacteroids.

Publications: 84/07 to 87/12
MCCLUNG, C.R., SOMERVILLE, J.E., GUERINOT,
 M.L. and CHELM, B.K. 1987. Structure of the
 Bradyrhizobium japonicum gene hemA encoding
 5-aminolevulinic acid synthase. Gene
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MCCLUNG, C.R. and CHELM, B.K. 1987. A genetic

locus essential for formate-dependent growth of Bradyrhizobium japonicum. J. Bacteriol. 169:3260-3267.

23.040* CRISO049430
GENETIC SELECTION-MANIPULATION TO ENHANCE
PREDATOR AND PARASITE EFFECTIVENESS

STEINER W M M; Parasite-predator Bio & Ecol Bio Control of Insects Lab; Agricultural Research Service, Columbia, **MISSOURI** 65211. Proj. No.: 3622-24000-006-00D

Project Type: INHOUSE Agency ID: ARS Period: 01 OCT 84 to 30 SEP 89

Objectives: Genetically map and characterize chromosomal, morphological, DNA, and allozyme variants of selected entomophagous insects and determine their physiological, developmental

and behavioral significance and their potential for utilization in bioengineering programs to enhance entomophageefficiency.

Approach: Populations of selected entomophages will be assayed using classical gene- tic techniques and restriction enzyme (DNA) technology to establish gene- tically typed isolines. These will be used to establish linkage maps and study genome structure in the context of predator-parasite/prev models. About 80 % of effort will be devoted to the model offered by Heliothis zea and its entomophages with 20% of effort reserved to develop genetic know- ledge of other models. Selected variants will also be biochemically char- acterized and their distributions in natural populations determined. Lab- oratory tests of physiological, developmental and behavior response under stresses posed by pesticides, temperature and desiccation will reveal whichphenotypes can enhance natural population fitness characteristics. Methods of transferring desirable traits will be developed.

Progress: 88/01 to 88/12. Selection for insecticide resistance in females of the parasitoid Microplitis croceipes was initiated to determine how amenable this parasite of Heliothis spp. is for development of insecticide resistant varieties. After 5 generations of inbreeding, two of five lines demonstrated an increase in LD values, going from 0.82 ug fenvalerate (in 0.5 ul acetone topical application) to a 16-fold increase at 13.12 ug with an accompanying increase in number of males being produced and a decline in line fertility. Selection ceased at the 6th round when the lines were lost due to the increased selection pressure. The observed selection response is a typical one seen for braconid wasps, and suggests this is the upper limit to which the M. croceipes genome may respond to insecticide selection. In other studies, three species of nabids were found to segregate for allozyme variation at an esterase locus (EST-1!) and an adenylate kinase locus (ADK-3). These were not in genetic equilibrium and reduction in the numbers of heterozygotes in nature suggests strong population subdivision. These loci may serve a diagnostic function to differentiate closely related nabid species.

Publications: 88/01 to 88/12

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23.041 CRISO034200 GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN

POLACCO J C; Biochemistry; University of Missouri, Columbia, MISSOURI 65211. Proj. No.: MO-00019-1 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 84 to 31 AUG 86

Objectives: PROJ 8400505 Recover a soybean seed urease cDNA clone and use it to determine the amount and specificity of urease transcript in cotyledons, leaves and suspension cultures of normal and urease-negative soybeans. Develop the genetic basis of urease-nulls. Explore genetic, nutritional and environmental influences on urease synthesis.

Approach: Identify cloned urease byhybridization to two separate synthetic oligonucleotides. Dot blots, \$1 mapping and nuclear run-off transcripts will determine the levels, synthetic rates and type of urease transcripts in various tissues. Analyze revertants, gene dosage effects and allelism with structural gene variants. Screen for new urease nulls. Nutrition and environment will be altered in cultures of cotyledons and suspended cells of normal and urease-negative varieties.

Progress: 84/09 to 86/08. Mutations affecting each of the 2 urease isozymes were recovered in M2 populations of EMS and NMU-treated soybeans. Four mutants allelic with sun (seed urease-null), the lesion responsible for lack of the embryo-specific urease in PI 229324, were recovered. Two have phenotypes indistinguishable from sun, i.e. no detectable embryo-specific urease mRNA or protein. However, two others are leaky. One, n8, appears to accumulate <1% the urease protein of normal seeds. However, the enzyme appears normal by kcat, pH-dependence, heat-stability and aggregation state. The second mutant, n6, makes higher levels of an altered protein: low kcat, increased heat lability, aberrant pH-dependence and aggregation states. Thus sun appears to $\,$ encode the embryo-specific urease since mutations at sun affect both the level and nature of the urease gene products. We propose that sun and the closely linked Eu locus, which controls urease aggregation state, are actually in the same locus. Urease genomic and cDNA (embryo) has been cloned and partially sequenced. The gene appears to be highly disrupted by introns and so far shows 83% amino acid homology to the sequence determined for isolated Jackbean urease. cDNA and genomic comparisons are underway to assign the genomic clone to one of two urease isozymes, the embryo-specific or the ubiquitous. RFLP's have been found and are being mapped relative to sun.

Publications: 84/09 to 86/08
START, W.G., YU, M., POLACCO, J.C.,
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ALTSCHULER, M. Two Soybean Seed
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Levels of Lipoxygenase Transcripts. Plant
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WINKLER, R.G. August 86. Ureide Catabolism in
Nitrogen Fixing Soybean Plants. Ph.D.
Dissertation. University of Missouri.

MEYER-BOTHLING, L.E. December 86. Soybean Mutants Aberrant in Expression of the Urease Isozymes. Masters Thesis. University of Missouri.

23.042 CRISOO81616 GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES

POLACCO J C; Biochemistry; University of Missouri, Columbia, **MISSOURI** 65211.

Proj. No.: MO-00019 Project Type: HATCH Agency ID: CSRS Period: 01 JUL 85 to 30 JUN 90

Objectives: Since the soybean urease isozymes (ubiquitous and embryo-specific) are differentially regulated and since the ubiquitous form is likely involved in nitrogen fixation we propose to: isolate the structural gene(s) for the embryo-specific and ubiquitous urease isozymes; identify metabolic signals on urease synthesis, induce and select mutations in structural and regulatory genes for each urease, and use cloned urease genes as probes to study the molecular bases of urease regulation and of mutants with altered urease production.

Approach: A urease genomic clone is being used to recover and identify ubiquitous and embryo-specific genes. These will be used to study transcriptional control in cell culture and in both mutant and normal intact plants. Several urease mutants have been recovered.

Progress: 88/01 to 88/12. A fourth urease locus (eu4) has been identified in sovbean. A mutation (eu4-aj3) at this locus eliminates urease activity but not antigen in soybean leaves. Roots and callus culture in the eu4-aj3 mutant contain 100 and 40%, respectively, the urease activity of the Williams 82 progenitor. We are testing the hypothesis that eu4 encodes a ubiquitous urease isozyme which is the exclusive species of leaf. These tests include comparison of urease RFLP segregation and the eu4-aj3 trait, and transient expression assay of urease genes of urease in eu4-aj3 leaf propotoplasts. The urease-negative phenotypes of eu2 and eu3-e1 (lacking urease in all vegetative tissue) were shown to be tissue autonomous in graft experiments. The eu1 (embryo-specific urease locus), eu2 and eu3 loci showed no cosegregation with RFLP's revealed by urease clone E15, corroborating deduced amino acid sequence data indicting that E15 does not encode the embryo-specific urease.

Publications: 88/01 to 88/12
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nitrogen-fixing legumes. TIBS 13:97-100.
HOLLAND MA, JD GRIFFIN, LE MEYER-BOTHLING, JC
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23.043 CRISO131842 STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES

STASWICK P E: Agronomy: University of Nebraska, Lincoln, **NEBRASKA** 68583.

Proj. No.: NEB-12-164 Project Type: CRGD Agency ID: CRGO Period: O1 JUL 87 to 30 JUN 90

Objectives: PROJECT 8700137. The overall purpose of the proposed research is to understand the molecular biology of soybean (Glycine max L. Merr.) leaf storage proteins (SLSP). Specific goals are to better understand the structure and genetic relatedness of these coordinately expressed genes, the level at which leaf storage protein gene expression is controlled, and the nature of the signal(s) which modulate the expression of these genes.

Approach: The experimental approach will be to determine the nucleotide of SLSP cDNA clones, isolate and characterize the SLSP genes, monitor changes in SLSP mRNA levels during plant development by RNA blot hybridizations, identify hormonal, nutrient and developmental events which affect the expression of SLSP genes at the mRNA level.

Progress: 88/01 to 88/12. The goal of this project is to isolate, characterize and determine factors which regulate the expression of the genes for soybean vegetative storage proteins. cDNA clones have been isolated and sequenced. They have been used to demonstrate that expression of the genes is highly regulated in leaves in response to the demand for mobilized reserves by plant sinks. Jasmonic acid induces vegetative storage protein gene expression in leaves and in soybean suspension cell cultures. An antibody assay (ELISA) has been developed to determine endogenous levels of jasmonic acid in plants. The storage protein genes are being isolated and characterized.

Publications: 88/01 to 88/12
STASWICK, P. (1988). Soybean vegetative
 storage protein structure and gene
 expression. Plant Physiol. 87:250-254.

23.044 CRISO059127 GENETICS AND BREEDING OF SOYBEANS

KIANG Y T; Plant Science; University of New Hampshire, Durham, **NEW HAMPSHIRE** 03824.

Proj. No.: NH00211 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: To investigate genetic variation in wild soybean populations and cultivated soybean germplasm. To use isozyme and RFLP markers to construct the genetic map of soybean chromosomes. To develop soybeans adapted to the Northeast and suitable for table use.

Approach: Wild soybean and cultivated soybean germplasm will be examined for enzyme variation (24 enzymes and one protein) by horizontal polyacrylamide and starch gels eclectophoresis. Inheritance of new variants will be studied by examining segregation of F(2) and F(3)generations. Amount of genetic variation will be estimated by calculating heterozygosity, number of alleles per locus, and polymorphism. DNA isolated from seedlings will be digested by a variety of restriction endonucleases. DNA fragments will be separated, and blotted onto nylon membranes for probing specific sequences. These markers, along with isozyme and morphological markers, will be used to construct chromosome genetic maps. A wide range of soybean germplasm with high yield and early maturity will be used to develop a high yield early lines suitable for vegetable use in the Northeast region.

Progress: 88/01 to 88/12. Genetic analysis of endopeptidase isozyme variants in soybean showed that the three variants were controlled by a single locus (Enp-a, Enp-b, Enp-c). Inheritance of phosphoglucose isomerase isozyme variants in soybean were controlled by three loci (Pgil, Pgi2, Pgi3). Three alleles at the Pgil (Pgil-a, Pgil-b, Pgil), two alleles at the Pgi2 (Pgi2, pgi2), and two alleles at the Pgi3 locus (Pgi3-a, Pgi3-b) were observed. PGI enzymes are dimers, and both inter-and intralocus heterodimers were observed. Pgil and Pgd1 (6-phosphogluconate dehydrogenase locus 1) were linked with recombination frequency of 15 +/- 0.7% betweent the two loci. The two loci. The two loci were assigned to linkage group 16.

Publications: 88/01 to 88/12

DOONG, J.Y.H. and KIANG, Y.T. 1987. Inheritance and property of soybean endopeptidase. Biochem. Genet. 25:845-852.

endopeptidase. Biochem. Genet. 25:845-852. CHIANG, Y.C. GORMAN, M.B. and KIANG, Y.T. 1987. Inheritance and linkage analysis of phosophoglucose isomerase isozymes in soybeans. Biochem. Genet. 25:893-900.

DOONG, J.Y.H. and KIANG, Y.T. 1987. Inheritance of aconitase isozymes in soybean. Genome 29:713-717.

CHIANG, Y.C. and KIANG, Y.T. 1987.
Inheritance and linkage relationships of 6-phosphogluconate dehydrogenase isozymes in soybean. Genome 29:786-792.

KIANG, Y.T. and CHIANG, Y.C. 1988. Mapping the B-amylase locus (Am3) on soybean linkage group 1 Chromosome. J. Heredity 79:107-109.

KIANG, Y.T., CHIANG, Y.C., DOONG, J.Y.H. and GORMAN, M.B. 1988. Genetic variation of soybean germplasm. In S.C. Hsieh (ed.) Crop Exploration and Utilization of Genetic Resources. pp. 93-99.

23.045 CRISO088984
GENETIC STRUCTURE OF CULTIVATED SOYBEANS AND
THE WILD SOYBEAN (GLYCINE SOJA) GERMPLASM

KIANG Y T; Plant Science; University of New Hampshire, Durham, **NEW HAMPSHIRE** 03824. Proj. No.: NH00284-SProject Type: SPECIAL GRANT Agency ID: CSRS Period: 01 AUG 82 to 31 JUL 87

Objectives: To estimate genetic variation of Glycine max (L.) Merr. and Glycine soja Sieb. &Zucc. To compare genetic structure of soybean germplasm (G. max & G. soja) in relation to geographic origins. To determine genetic linkage in soybeans. To determine association of genetic variation and physiological, morphological and agronomic traits of Glycine soia.

Approach: 16 seed proteins and enzymes will be examined by gel elcetrophoresis. The inheritance of variants will be studied by examining the F(1), F(2) and F(3) seeds. Genetic structure of different geographic origins will be compared by polymorphism. F(2) and F(3) seeds of various crosses involving different enzymes variants will be examined for their genetic linkage by gel electrophoresis. Morphological and agronomic traits of different sources of G. soja will be measured in the greenhouse and field and their seed enzymes will be examined by gel electrophoresis to study the association of agronomic traits and genetic variation.

Progress: 86/01 to 86/12. The soybean seed leucine aminopeptidase locus (Lap1) was found to be linked to the Kunitz trypsin inhibitor locus (Ti) with a recombination frequency of 15.3plus or minus0.9 percent. The two loci are in linkage group 9. Both Lap1 and Ti loci are inherited independently of the flower color locus (W1) in linkage group 8. Three protein loci Ap, Lap1 and Ti were mapped on soybean linkage group 9 chromosome. The recombination frequency between the Ap and Lap1 is 23.6plus or minus1.0%, and that between the Ap and Ti loci is 6.6plus or minus0.5%. The map distance between the Lap1 and Ti loci is 16.3plus or minus1.0 map units. The order of the three loci is Ap-Ti-Lap1. The linkage relationship between the alcohol dehydrogenase 1 (Adh1) and the flower color locus (W1) were examined. The two loci are linked with a recombination value of 20.6plus or minus1.2%. They are members of linkage group 8. The isozyme study showed the Korean germplasm of both soybean species having more genetic variation than other geographical sources examined, and the wild soybean germplasm contains much more genetic variation than the cultivated soybeans.

Publications: 86/01 to 86/12
KIANG, Y.T. and CHIANG, Y.C. 1986. Genetic
linkage of a leucine aminopeptidase locus
with the Kunitz trypsin inhibitor locus in
soybeans. J. of Hered. 77:128-129.

DOONG, J.Y.H. 1986. An electrophoretic study on soybean isozymes. M.S.

Thesis. pp101. University of New Hampshire, Durham, NH.

23.046 CRISO022778 CONTROL OF THE BIOSYNTHESIS OF PROTEINS & AMINO AC IDS IN LEGUME SEEDS

THOMPSON J F; MADISON J T; Agricultural Research Service; Agricultural Research Service, Ithaca, **NEW YORK** 14853. Proj. No.: 1907-20170-001-00D

Project Type: INHOUSE

Agency ID: ARS Period: 12 JUN 72 to 01 NOV 86

Objectives: To understand the mechanisms that regulate methionine synthesis and to modify the structure of soybean storage protein genes in such a way as to increase the methionine content of the storage proteins or legume seeds and to insert the modified gene into soybean genomes.

Approach: Soybean tissue culture lines resistant to ethionine and containing an abnormally high level of free methionine are isolated. These high methionine lines are tested for activity and regulation of methionine biosynthetic enzymes and are assayed for levels of intermediates in the biosynthetic pathway. DNA clones having portions of soybean storage protein genes are prepared. These clones are used to isolate intact storage protein genes which are prepared. These clones are used to isolate intact storage protein genes which are characterized as to sequence, introns, exons, 3' and 5' noncoding regions. Knowing the structure of the gene, the gene will be modified in appropriate places (to increase coding for methionine) and the gene will be inserted into the soybean genome.

Progress: 86/01 to 86/03. The major effort has been applied to understanding why exogenous methionine increases the protein methionine of cultured soybean seeds by one-fifth. It has previously been established that methionine eliminates the beta subunit of the 7 S storage protein causing a decrease in the 7 S storage protein and an increase in the 11 S storage protein It was shown that the effect of methionine was not due to a more rapid degradation of the beta subunit but was due to the absence of the beta subunit messenger RNA.

Publications: 86/01 to 86/03
HOLOWACH, L.P., MADISON, J.T. AND THOMPSON,
J.F. 1986. Studies on the mechanism of the
regulation of the mRNA level for a soybean
storage protein subunit by exogenous
L-methionine. Plant Physiol. 80:561-567.

23.047 CRISO093865 MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE)

DOYLE J J; L. H. Bailey Hortorium; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-187405 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 91

Objectives: The purpose of this research is to elucidate phylogenetic relationships of the soybean (Glycine max) and its wild relatives by the application of molecular techniques. Specific problems include: the relationship of

the soybean to its wild progenitor, G. soja phylogenetic affinities of wild perennial soybean species, origin and evolution of polyploid complexes in perennial species, and establishing the relationships between the annual and perennial subgenera.

Approach: A variety of DNA sequences will serve as markers for the evolutionary studies proposed. These include the tandemly repeated multigene family encoding the 18S and 25-28S ribosomal RNAs, the 5S rRNA repeat, and chloroplast DNA. Techniques will include restriction endonuclease mapping of genomic DNA, molecular cloing of specific genes, and DNA sequencing. Data will be analyzed and phylogenies reconstructed by cladistic and distance methods.

Progress: 88/01 to 88/12. Over the past year, a number of projects concerning the evolution of the soybean and its relatives (Glycine) have been completed. These have included surveys of a gene duplication and a chloroplast DNA (cpDNA) deletion in the legume family as a whole, and a large cpDNA inversion in the legume tribe Phaseoleae. A phylogeny of Glycine based on chloroplast DNA restriction site variation has been completed, indicating the existence of three major clades within the perennial subgenus. These evolutionary lineages are in agreement with previous data from crossing studies, and indicate that molecular and more traditional systmatic approaches converge on similar answers in this group. The evolution of the G. tabacina and G. tomentella polyploid complexes has been studied using nuclear ribosomal genes and cpDNA. Fixed hybridity for 5S and 18S-25S nuclear ribosomal RNA genes among polyploids has provided insights into their alloployploid origin. Chloroplast DNA variation among diploid genome donors has led to precise hypotheses of materinal progenitors of several polyploids. Considerable cpDNA polymorphism has been found in several diploid and polyploid species of the subgenus. Germplasm from several newly described species has been obtained, and the cpDNAs of these species have been characterized and into phylogenetic schemes for the genus.

Publications: 88/01 to 88/12

DOYLE, J.J. 1988. 5S Ribosomal gene variation in the soybean and its progenitor.
Theoretical and Applied Genetics 75:621-624.

DOYLE, J.J. and DOYLE, J.L. 1988. Natural interspecific hybridization in eastern North American Claytonia. American Journal of Botany 75:1238-1246.

DOYLE, J.J. and BROWN, A.H.D. 1988. 5S nuclear ribosomal gene variation in the Glycine tomentella polyploid complex (Leguminosae). Systematic Botany (in press).

WEEDEN, N.F. and DOYLE, J.J. and LAVIN, J. 1989. Distribution and evolution of a glycosephosphate isomerase duplication in the Leguminosae. Evolution (in press).

DOYLE, J.J. and DOYLE, J.L. and BROWN, A.H.D. 1989. A chloroplast DNA phylogeny of the wild perennial relatives of soybean (Glycine subgenus Glycine): congruence with morphological and crossing groups. (submitted)

DOYLE, J.J. and DOYLE, J.L. and GRACE, J.P. and BROWN, A.H.D. 1989. Reproductively isolated polyploid races of Glycine tabacina (Leguminosae) have different maternal progenitors (submitted).

LAVIN, M. and DOYLE, J.J. and PALMER, J.D. 1989. Evolutionary significance of the loss of the chloroplast DNA inverted repeat in the Leguminosae subfamily Papilionoideae. (submitted).

23.048 CRISOO88813 GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANDII

BISHOP P E; Microbiology; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695. Proj. No.: NCO5531 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 82 to 30 NOV 83

<code>Objectives:</code> Isolate Nif mutants defective for both the molybdo-enzyme N(2) fixation system and the recently discovered alternative system. Characterize these mutants using both biochemical and genetics techniques. Clone restriction endonuclease-generated fragments of chromosomal DNA containing nif genes. Characterize the cloned nif DNA using restriction, marker resaue and DNA sequence analysis.

Approach: Ethylmethane sulfonate (EMS) and Tn5 mutagenesis procedures will be employed in the isolation of Nif mutant strains of A. vinelandii. The Nif mutant strains will be characterized using high resoulution 2-dimensional gel electrophoeesis, by titration and extracts prepared from mutant cells with purified nitrogenase components, and by genetic mapping. Genes for the conventional molybdo-enzyme and alternative N(2) fixation systems will be cloned into Escherichia coli.

Progress: 85/01 to 85/12. Deletion strains of A. Vinelandii which are missing the structural genes (nifHDK) have been used to provide conclusive evidence for the presence of an alternative Nitrogen fixation system in this organism. An A. vinelandii nifH probe has been used to identify clones containing nifH-like sequences in an EMBL-3 genomic library constructed from a nifHDK deletion strain (CA11). The partial base sequence of one of these nifH genes has been determined. The alternative nitrogenase proteins have been partially purified from strain CA11.6 (carries nifHDK deletion and is tungsten tolerant). Isolation of a transposon mutant containing Tn5 inserted in nifB (required for FeMo-co synthesis) has facilitated the cloning of a 3.6-kb EcoR1 fragment containing the nifB gene. Since this gene is required for functioning of the alternative Nitrogen fixation system, sequencing this gene should provide further insight into the genetics of this system.

Publications: 85/01 to 85/12

BISHOP, P.E., RIZZO, T.M. AND BOTT, K.F. 1985. Molecular cloning of nif DNA from Azotobacter vinelandii. J. Bactériol. 162-21-28.

- SMITH, B.E., et al. R. 1985. The iron-molybdenum cofactor of nitrogenase.
- 597-603. In Nitrogen Fixation Research Progress, Martinus Nijhoff Publishers, Boston. 731 pp.
- KENNEDY, C., et al. 1985 Genetic and physical studies of nif and ntr genes in Azotobacter chroococcum and A. vinelandii. pp. 469-476. In Nitrogen Fixation Research Progress, Martinus Nijhoff Boston. 731 pp.
- BISHOP, P.E. AND EADY, R.R. 1985. Nitrogen fixation by a nifHDK deletion strain of Azotobacter vinelandii. p. 622. Nitrogen Fixation Research Progress, Martinus Nijhoff, Boston. 731 pp.

23.049 CRISOO91368 GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANDII

BISHOP P E; Microbiology; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO5531 Project Type: STATE Agency ID: SAES Period: O1 OCT 82 to 30 SEP 87

Objectives: Isolate Nif- mutants defective for both the molybdo-enzyme N2 fixation system and the recently discovered alternative system. Characterize these mutants using both biochemical and genetics techniques. Clone restriction endonuclease-generated fragments of chromosomal DNA containing nif genes. Characterize the cloned nif DNA using restriction, marker rescue and DNA sequence analysis.

Approach: Ethylmethane sulfonate (EMS) and Tn5 mutagenesis procedures will be employed in the isolation of Nif mutant strains of A. vinelandii. The Nif- mutant strains will be characterized using high resolution 2-dimensional gel electrophoresis, by titration of extracts prepared from mutant cells with purified nitrogenase components, and by genetic mapping. Genes for the conventional molybdo-enzyme and alternative N2 fixation systems will be cloned into Escherichia coli.

Progress: 82/10 to 87/09. Deletion strains of Azotobacter vinelandii lacking the structural genes for conventional Mo-containing nitrogenase were shown to fix nitrogen under Mo-deficient conditions. This provided definitive proof for the existence of an alternative nitrogen fixation system in this diazotroph. An alternative nitrogenase was purified from one of the deletion strains. This nitrogenase is expressed only in the absence of V and Mo and does not contain either element. Genes thought to encode this nitrogenase have been cloned and partially sequenced. Transcripts from these genes are repressed by both V and Mo. Mutants (Tn5-mediated) which are unable to express the alternative nitrogen fixation system have been isolated and partially characterized. The wild-type allele for one of these mutations has been cloned on a 3.8-Kbp EcoR1 fragment of DNA. This entire fragment has been sequenced and shown to contain the nifB-nifQ region of the A vinelandii genome. Northern blot analysis using probes from this region indicate that it is a nif operon which is expressed in the presence or absence of Mo or V. However, the operon is repressed by ammonia. Insertion mutations in this operon indicate that nifB is required for nitrogen fixation under all conditions but nifQ is not required for normal functioning of the alternative nitrogenase system.

Publications: 82/10 to 87/09 NO PUBLICATIONS REPORTED THIS PERIOD.

23.050 CRISO132499 CHARACTERIZATION OF AN ALTERNATIVE NITROGEN FIXATION SYSTEM IN AZOTOBACTER VINELANDII

BISHOP P E; Microbiology; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO5650 Project Type: STATE Agency ID: SAES Period: 01 OCT 87 to 30 SEP 92

Objectives: Sequence both nifH-homologous and flanking DNA sequences which have been cloned from the nifHDK deletion strain CA11 and analyze the sequences for ORF's and promoter regions. Sequence cloned DNA's which contain wild-type alleles for Tn5 mutations that confer a Nif-phenotype under Mo-deficient conditions. Analyze the sequences for ORF's and promoter regions. Use DNA's that are specific for ORF's identified in objectives nos. 1 and 2 to probe RNA transcripts made under Mo-deficient conditions. Characterize strains carrying site-specific deletions in the ORF's identified under objectives nos. 1 and 2. Continue purification and characterization of nitrogenase proteins which are expressed by the deletion strain under Mo-deficient conditions.

Approach: DNA sequencing using the dideoxy chain termination method will be used to analyze the genes involved in the alternative N(2) fixation system. Functional genes will be identified by generating mutations with Tanr cartridges. Site specific mutations will also be created using synthetic oligonucleotides. Transcription will be studied using Northern blot techniques. Alternative nitrogenase proteins will be characterized by EPR, EXAFS and neutron activation analysis.

Progress: 88/01 to 88/12. The nucleotide sequence of a region of the Azotobacter vinelandii genome exhibiting sequence similarity to nifH has been determined. The order of open reading frames (ORFs) within this 6.1-kbp region was found to be anfH alternative nitrogen fixation (nifH-like gene), anfD (nifD-like gene), anfG (potentially encoding a protein similar to the product of vnfG and A. chroococcum), anfK (nifK-like gene) followed by two additional ORFs. The 5'-flanking region of anfH contains a nif promoter similar to that found in the A. vinelandii nifHDK gene cluster. The presumed products of anfH, anfD and anfK are similar in predicted Mr and pI to the previously described subunits of nitrogenase-3. Deletion plus insertion mutations introduced into the anfHDGK region of wild-type strain A. vinelandii CA resulted in mutant strains that were unable to grow in Mo-deficient N-free medium, but grew in the presence of 1 uM

Na2MoO4 or V205. Introduction of the same mutations into the nifHDK deletion strain CA11 resulted in strains that grew under diazotrophic conditions only in the presence of vanadium. The lack of nitrogenase-3 subunits in these mutant strains was demonstrated through two-dimensional gel analysis of protein extracts from cells derepressed for nitrogenase under Mo and V deficiency. These results indicate that anfH, anfD and anfK encode structural proteins for nitrogenase-3.

Publications: 88/01 to 88/12

BISHOP, P. E. et al. 1988. Alternative nitrogen fixation systems in Azotobacter vinelandii. 1988. In: Nitrogen Fixation: Bothe, H., F. (eds.), Gustar Fisher Berlag, Stuttgart-New York. p. 71-79.

JOERGER, R. D., and P. E. BISHOP. 1988. Bacterial alternative nitrogen fixation systems. 1988. CRC Critical Reviews in Microbiology 16:1-14.

JOERGER, R. D. AND P. E. BISHOP. 1988. Nucleotide sequence and genetic analysis of the nifB-nifQ region from Azotobacter vinelandii. J. Bacteriol 170:1475-1487.

23.051 CRISO093970 STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREDII, A NEWLY DESCRIBED SPECIES OF SOYBEAN RHIZ

ELKAN G H; Microbiology; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.

Proj. No.: NCO3906 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 89

Objectives: To determine the nature of regulation of the nif and nod genes in Rhizobium fredii a new species of soybean rhizobia. To improve biological nitrogen fixation in soybeans by producing and selecting genetic hybrids of R. fredii and Bradyrhizobium japonicum for enhanced symbiotic properties. The long range goal is the development of improved strains of bacteria for use as a legume inoculant.

Approach: The following experiments will be done: isolate total DNA from R. fredii isolates- PRC 206, PRC 206C, PRC 194 and PRC 063, restriction endonuclease digestion of total DNA, Southern hybridization of DNA with labeled pRmR2; isolation of plasmid DNA from PRC 063, restriction endonuclease digestion of plasmid DNA, Southern hybridization with pRmR2 comparing plasmid and total DNA; Northern blot analysis and nitrogenase enzyme level assays; and cloning and sequencing of some regions of chromosomal (integrated) and plasmid borne nif genes.

Progress: 88/01 to 88/12. Rhizobium fredii strain USDA 206 harbors four large plasmids, one of which carries nodulation and nitrogen fixation genes. A previously isolated group of plasmid cured derivatives of strain USDA 206 were compared with each other to determine possible functions for the plasmids. Mutant strain 206CANS was isolated as a non-mucoid derivative of strain 206CA, a mutant cured of two plasmids. The non-mucoid phenotype of

206CANS was only expressed when the strain was grown on certain media, particularly those with polyols as carbon sources. Plasmid pRj206b of strain 206CANS was previously shown to have a higher copy number than the same plasmid in strain USDA 206 and in strain 206CA. This plasmid, when transferred to non-mucoid strains, conferred on recipient strains a non-mucoid phenotype. The symbiotic effectiveness of the wild-type and cured strains was compared. Overall, few differences were shown, but strains 206CA and 206CANS were found to have higher nitrogenase activity than the other strains. Thus, there appears to be a possible relationship between colony morphology, plasmid copy number, and symbiotic effectiveness.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

23.052 THE ROLE OF CERCOSPORIN IN PATHOGENICITY AND THE REGULATION OF ITS SYNTHESIS

UPCHURCH R G; Plant Pathology; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.

Proj. No.: NCO5636 Project Type: STATE Agency ID: SAES Period: 10 JAN 86 to 30 SEP 91

Objectives: Isolate and characterize cercosporin-minus mutants, develop a genetic transformation system for C. Kikuchii using plasmid vectors, isolate genes encoding cercosporin regulation and biosynthesis, determine roles of cercosporin in plant disease, determine and analyze the nucleotide sequence of these genes, and analyze the effects of cultural conditions, including exposure to plant molecules from susceptible and resistant cultivars, on gene expression by transcription studies.

Approach: A combination of molecular biology and biochemistry will be used to characterize mutants (TOX) and to clone toxin genes by complementing the mutants. Genes characterized by sequencing will be used in regulation studies. The role of toxin in disease will be studied by use of TOX mutants and genetically restored (TOX) mutants with plant inoculation experiments.

Progress: 88/01 to 88/12. Seven Cercospora-kikuchii UV induced mutants have been isolated and partially characterized with respect to virulence on the soybean plant host. protein profiles with one dimensional SDS-PAGE gels, and cercosporin synthesis. Mutants that do not synthesize detectable toxin in laboratory media do not incite plant lesions. This finding indicates that toxin is necessary for lesion production. One of these toxin-minus mutants produces a brilliant yellow pigment, perhaps a cercosporin biosynthetic pathway intermediate. Studies are underway to determine its identity and structure. The benA gene has been cloned from a benomyl resistant mutant of C. kikuchii. This gene has been incorporated as the fungal marker gene in the construction of a Cercospora genetic transformation vector,

pGU18. Use of this vector in transformation protocols involving either polyethylene glycol treated or electroporated fungal protoplasts have given low frequency transformation rates. Work continues with the aim of increasing the frequency. An abundant (12%) 72kD protein has been identified in a toxigenic parental strain of C. kikuchii. The protein appears to be light regulated, and regulated concomitantly with cercosporin synthesis.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

23.053 CRISO099732 CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING DIHYDROFOLATE REDUCTASE

FREISHEIM J H; Biochemistry; Medical College of Ohio, Toledo, **OHIO** 43699.

Proj. No.: OHOR-8601675 Project Type: CRGO Agency ID: CRGO Period: 15 AUG 86 to 31 AUG 88

Objectives: PROJECT 8601675. To provide a rational approach to herbicide design using folate antagonist inhibitors of plant dihydrofolate reductase (DHFR). Specific objectives: Purification of soybean seedling DHFR; Protein sequencing of amino-terminal region of soybean DHFR and derived CNBr fragments; Cloning of soybean seedling DHFR gene; Sequencing of a full-length cDNA coding for soybean DHFR.

Approach: The protein sequence information will be used to construct oligodeoxy-nucleotides as probes for corresponding regions of the cDNA. Other approaches include immunoprecipitation of polysomes using an antibody preparation known to significantly cross-react with the plant enzyme.

Progress: 88/01 to 88/08. Soybean seedling dihydrofolate reductase (DHFR) has been isolated and purified to apparent homogeneity using methotrexate (MTX) affinity chromatography, gel filtration and Blue Sepharose chromatography. This plant enzyme has a molecular weight of ca. 22,000, similar to that of the vertebrate and bacterial enzymes. Recent studies have also shown that amino acid sequence-specific antibodies to intact human DHFR and also antibodies to CNBr-generated fragments of human DHFR bound to the soybean enzyme on Western blots and cross-reacted significantly (70%) in immunoassays. The specific activity of the soybean enzyme is 15 units/mg, with a pH optimum of 7.4. K(m) values of the enzyme for dihydrofolate and NADPH are 17 and 30 mu M, respectively. Unlike other eukaryotic DHFRs the plant enzyme showed no activation with organomercurials and was inhibited by KC1 and urea. The affinity of the enzyme for folate is relatively low (I(50) =130 mu M) while methotrexate binds very tightly (K(D)<10 M). Pyrimethamine binding to the plant enzyme was weaker, while trimethoprim binding was stronger than to vertebrate DHFR. Using antibodies to DHFR we have screened a gamma gtll soybean seedling expression library. Rescreening positive plaques using the antibodies has resulted in four positive

plaques. We have now sequenced a full-length cDNA which encodes soybean seedling DHFR. We are now in the process of trying to obtain high expression levels of this plant enzyme using a plasmid vector construct using an E.

Publications: 88/01 to 88/08

RATNAM, S., DELCAMP, T.J. and FREISHEIM, J.H. 1986. Dihydrofolate Reductase from Soybean Seedlings. Eighth International Symposium on Pteridines and Folic Acid Derivatives: Chemical, Biological and Clinical Aspects.

RATNAM, S., DELCAMP, T.J. and FREISHEIM, J.H. 1986. Dihydrofolate Reductase for Soybean Seedlings. Chemistry and Biology of Pteridines. Ed. by B.A. Cooper and V.M. Whitehead, Walter de Gruyter, Berlin, New York. 815-818.

RATNAM, S., DELCAMP, T.J. and FREISHEIM, J.H. 1987. Purification and Characterization of Dihydrofolate Reductase from Soybean Seedlings. Arch. Biochem. Blophys 225:279-289.

23.054* CRISO137399
RFLP AND MOLECULAR ANALYSIS OF ROOT KNOT
NEMATODES. NEMATODE INFECTED PLANTS AND PEACHES

ABBOTT A G; BILLARD R; LEWIS S A; Biological Sciences; Clemson University, Clemson, **SOUTH CAROLINA** 29634.

Proj. No.: SC01310 Project Type: HATCH Agency ID: CSRS Period: O1 FEB 89 to 31 DEC 92

Objectives: Generate DNA clones for the construction of RFLP (restriction fragment length polymorphism) maps in peach and for race identification in nematodes; cDNA clones of nematode infected plant tissue will be used to search for plant genes induced during giant cell formation.

Approach: Peach RFLP maps will be constructed using random genomic clones in pUC8 and polymorphisms detected in hybrid peach X almond crosses. Random clones of the nematode species M. ingognita will be screened for polymorphism in biotypes 1, 2, 3 and M. areneria biotypes 1 and 2. Cascade hybridization will identify clones unique to M. incognita infection of soybean tissue. These will be studied in the hypersensitive response.

23.055 CRISO099966 ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES

FOLK W R; Microbiology; University of Texas, Austin, TEXAS 78712.

Proj. No.: TEXR-8601744 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 86 to 30 JUN 88

Objectives: Project 8601744. tRNAs are critical elements in the synthesis of all plant proteins, yet virtually nothing is known about the organization and expression of plant nuclear tRNA genes. We have isolated the genes for tRNAmet and tRNAtrp from the soybean

genome. We propose the following objectives: We will measure the expression of these tDNAs in developing plant tissues and in plants subjected to nutritional deprivation. We will develop expression systems so as to study synthesis of tRNAs in vivo from newly introduced tDNAs and measure the effect upon protein synthesis of altering the tRNA gene dosage. These experiments should provide new insights into how plants express their genetic information, and may provide the means to genetically alter the amounts of nutritionally important plant proteins.

Approach: The approaches we will employ in these studies include RNA hybridization, DNA hybridization, plant transformation by Agrobacterium vectors.

Progress: 88/01 to 88/12. We cloned and sequenced the tRNAmet gene from soybean. This gene functions in vivo to express a tRNAmet. We have introduced the gene into tobacco, and are attempting to measure its expression. We are developing transient expression assays with tobacco protoplasts so as to be able to detect changes in expression resulting from modification of the 5' flanking sequences. We have measured the tRNAmet gene copy number in a variety of soybean tissues at different stages of development. Despite its low redundancy, we find no evidence for gene amplification.

Publications: 88/01 to 88/12
PALMER, J. and FOLK, W. 1987. Isolation and
 sequence analysis of a nuclear tRNA_m_e_t
 gene from soybean. Plant Molecular Biology
 8 47-51. (Other manuscripts are in
 preparation).

23.056 CRISO140905 GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS

PALMER R G; SHOEMAKER R C; LARK G A; University of Utah, Salt Lake City, **UTAH** 84112.

Proj. No.: 3625-21000-001-01\$

Project Type: COOPERATIVE AGREE. Agency ID: ARS Period: O1 OCT 85 to 30 SEP 89

Objectives: To construct a chromosomal linkage map in soybean using restriction fragment length polymorphisms with DNA markers.

Approach: DNA will be extracted from leaf tissue of two parental soybean lines and examined on Southern blots for polymorphisms using a number of restriction enzymes. A genomic library of soybean will be screened to isolate random clones which represent single or low copy sequences. These will be used asprobes in the Southern blotting experiments to determine whether they can serve as markers to distinguish between the two parental varieties. Once a collection of markers has been obtained, the markers will be mapped with respect to one another and to other morphological plant traits by examining their segregation patterns in backcrossed plant material. Additionally, a somatic approach to mapping may be undertaken by examining the behavior of markers during chromosome loss induced in tissue culture.

Progress: 88/01 to 88/12. We are preparing a RFLP map involving a G. max cross - 'Minsoy' x 'Noir 1'. Probes cloned into pBS with PstI continue to be isolated. By now, over 400 clones have been examined yielding ca. 80 RFLP markers for this G. max cross. Fifty-five of these have been mapped in a F2 population grown in Utah. DNA from this population was exhausted and a new population of plants was prepared last summer. DNA has now been isolated from more than 80 new F2 plants from this population. These preparations will be used to continue mapping these markers and polymorphisms (9 available) identified from a cDNA library by B. Matthews. Tissue cultures are being prepared from the same new F2 plants. Previous work has shown that RFLP markers do not change in cultures prepared from stems, cotyledons or leaves. Suspension cultures can be preserved in liquid nitrogen and provide an unlimited source of DNA for mapping RFLP markers. At present we have DNA from 11 such cultures and 20 more suspension cultures are available. If the project is completed we will have a total of ca. 100 suspension cultures derived from 100 F2 plants. These can be used, in the future to map RFLP markers until a saturated map is obtained. Parallel to these, Dr. Levi Mansur (at ISU) is advancing seed from the same F2 population to prepare more than 80 recombinant inbred lines, ultimately to be used for mapping quantitative traits as well as studying closely linked markers.

Publications: 88/01 to 88/12

APUYA, N.R., FRAZIER, B.L., KEIM, P., RDTH, E.JILL and LARK, K.G. 1988.

Restriction fragment length polymorphisms as genetic markers in soybean, Glycine max (L.) Merrill. Theor. Appl. Genet. 75:889-901.

KEIM, P., DIERS, B.W., PALMER, R.G., SHDEMAKER, R.C., MACALMA, T. and LARK, K.G. 1989. Mapping the soybean genome with RFLP markers. Proc. IV World Soybean Res. Conf. Accepted March 12, 1988.

23.057 CHARACTERIZATION OF NITROGEN FIXATION GENES AND THEIR PRODUCTS FROM AZOTOBACTER VINELANDII

DEAN D R; Anaerobic Microbiology; Virginia Poly Inst, Blacksburg, **VIRGINIA** 24061. Proj. No.: VA-135143 Project Type: HATCH Agency ID: CSRS Period: O1 APR 86 to 31 MAR 91

Objectives: In this project we seek to achieve the following objectives: determine the complete nucleotide sequence of all the genes whose products are necessary for nitrogen fixation in Azotobacter vinelandii; overproduce each of these gene products in foreign hosts using recombinant DNA procedures; isolate mutants which are altered in each of the respective nitrogen fixation gene products; and reconstitute active nitrogenase using extracts from various mutant strains we have constructed.

Approach: Dur experimental approach to achieve the above goals will include DNA sequence analysis, overproduction of gene products in E.

coli, production of antibodies against purified proteins, site directed mutagenesis procedures, and spectroscopic analysis of purified proteins using X-ray absorption spectroscopy and Electron Paramagnetic Resonance.

Progress: 87/01 to 88/09. We determined nucleotide sequence of a 28,793 base-pair DNA fragment from A.vinelandii genome that includes and flanks nitrogenase structural gene region. This information was used to revise the previously proposed organization of major nif-cluster. The major nif-cluster from A.vinelandii encodes 15 nif-specific genes whose products bear significant structural identity when compared to corresponding nif-specific genes from K.pneumoniae.

Publications: 87/01 to 88/09
ND PUBLICATIONS REPORTED THIS PERIOD.

23.058 CRISO132901 ANALYSIS OF AZOTOBACTER VINELANDII N-FIXATION-SPECIFIC GENES AND THEIR PRODUCTS

DEAN D R; Anaerobic Microbiology; Virginia
Poly Inst, Blacksburg, **VIRGINIA** 24061.
Proj. No.: VA-428082 Project Type: CRGD
Agency ID: CRGO Period: O1 SEP 87 to 31 AUG 89

Objectives: Proj. 8701330. These experiments are aimed at determining the physiological ratio of the nitrogenase component proteins in the bacterium Azotobacter vinelandii. Further, we wish to determine the genetic mechanisms which operate in controlling component balance.

Approach: Various recombinant DNA procedure will be used to achieve the above goals. These procedures include: DNA sequence of the nif structural gene region and the some of the flanking regions, 51 and northern analysis of nif specific structural gene mRNA, testing for regulatory features of nif structural gene products using mutant strains and the examination of the physiological consequences of eliminating certain structural features within the nitrogenase gene cluster.

Progress: 87/01 to 88/09. The Azotobacter vinelandii nif-specific genes nifH, nifD, nifK, nifT, nifY, nifE, nifN, nifX, nifU, nifS, nifV, nifW, nifZ, nifM, nifF and nifA have been isolated and their nucleotide sequences determined. In the present project we have isolated mutant strains of A. vinelandii which have defined deletions, insertions or deletion plus insertions within each of the above nif-specific genes. Furthermore, we have identified a new set of genes whose products appear to be related to nitrogen fixation. These gene have not yet been identified in other organisms. This information was used to develop a genetic map of the major A. vinelandii nif gene cluster. Finally, nitrogenase component activities were measured in each of these mutant strains in an effort to identify the individual nif-specific functions of the respective gene products. Results of these experiments indicates that nifWZ are involved in maturation of the nitrogenase MoFe protein component, but are not involved in

CM 23

FeMo-co biosynthesis. The nifM gene product is involved in the maturation of the Fe protein component. No function has yet been established for the nifTYX genes. There are 4 graduate students involved in this project.

Publications: 87/01 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

CM 24 PEANUTS

24.001* CRISO089829 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT

PRING D R; CHDUREY P S; HIEBERT E; Plant Pathology; University of Florida, Gainesville, FLORIDA 32611.

Proj. No.: FLA-PLP-02317 Project Type: HATCH Agency ID: CSRS Period: 01 DCT 82 to 30 SEP 88

Objectives: Identification and Characterization of Agriculturally Important Genetic Systems. Regulation of Gene Expression and The Delivery of Genetic Material to Higher Plants and Associative Microorganisms. Somatic Cell Genetics and Plant Development: The Modification, Selection Regulation, and Propagation of Plants through Cell and Tissue Culture.

Approach: Plant viral genomes will be mapped and their products characterized. Recognition factors, both host and bacterial, will be characterized. Transposable elements of maize will be used as specific mutagens to identify rate limiting steps in starch biosynthesis. Plasmid-like DNAs in maize and sorghum will be tested as genetic vectors. Plant regeneration from protoplasts and callus will be attempted via organogenesis and somatic embryogenesis.

Progress: 83/10 to 88/09. Promoters and processing sites of maize mitochondrial atp6 are positioned 5' to Turf-13 and DRF221 in T cytoplasm maize, providing regulatory sequences associated with the 13 kD gene product of Turf-13. Maize nuclear backgrounds influence abundance of at least five transcripts associated with the gene. Abundance of the 13 kD gene product is reduced dramatically, while the Rf1 restorer only slightly reduces abundance of major transcripts, suggesting a role of the gene in translation. A maize cell suspension culture was used to study the biology and replication of mitochondrial DNA (mt DNA) and the two minicircular DNAs. All mt DNAs were synthesized rapidly during logarithmic growth phase, whereas no synthesis could be detected in stationary phase. The minicircular DNAs replicated earlier than the principal mt DNA. These data indicate that components of mitochondrial genome exhibit differential replication. Restriction digestion and Southern blot analyses of the bean golden mosaic virus (BGMV) isolated in Florida in comparisons with the Puerto Rican BGMV isolate revealed a high degree of sequence homology between the two isolates. However, distinct restriction patterns with four different endonucleases indicate that the isolates are not identical at the genomic level. A monoclonal antibody prepared to the Florida BGMV was useful in distinguishing some biological variants of BGMV.

Publications: 83/10 to 88/09

KENNELL, J.C., WISE, R.P. and PRING, D.R.

Influence of nuclear background on

transcription of a major mitochondrial

transcription of a maize mitochondrial region associated with Texas male sterile cytoplasm. Mol. Gen. Genet. 210:399-406. 1987.

PRING, D.R., GENGENBACH, B.G. and WISE, R.P. Recombination is associated with polymorphism of the mitochondrial genomes of maize and sorghum. Phil. Trans. Royal Soc. London B 319:187-198. 1988.

SMITH, A.G., CHDUREY, P.S. and PRING, D.R. Replication and amplification of the small mitochondrial DNAs in a cells suspension of Black Mexican Sweet maize. Plant Molec. Biol. 10: 83-90. 1987.

GILBERTSDN, R.L., FARIA, J.C., HIEBERT, E. and MAXWELL, D.P. 1988. Properties and cytology of bean golden mosaic in Brazil. Phytopathology, Abst. 441 submitted for the Annual Meetings of APS, San Diego, CA, Nov. 13-17, 1988.

CHANG, C.A., HIEBERT, E. and PURCIFULL, D.E. 1988. Characterization and immunological analysis of nuclear inclusions induced by bean yellow mosaic and clover yellow vein potyvirusus. Phytopathology 78, in press.

CHANG, C.A., HIEBERT, E. and PURCIFULL, D.E. 1988. Analysis of in vitro translation of the bean yellow mosaic virus RNA and the inhibition of proteolytic processing by antiserum to the 49K nuclear inclusion protein. J. gen. Virol.

HIEBERT, E. and DOUGHERTY, W.G. 1988.

Organization and expression of the vira.

24.002 CRISO138547 A STUDY OF GENETIC DIVERSITY IN PEANUTS USING CHLOROPLAST AND RRNA MOLECULAR MARKERS

HILU K W; Biology; Virginia Poly Inst, Blacksburg, **VIRGINIA** 24061. Proj. No.: VA-135292 Project Type: HATCH Agency ID: CSRS Period: O1 DCT 89 to 30 SEP 92

Objectives: The objectives of this are to: examine chloroplast and nuclear genetic diversity in cultivated peanut and fourteen related wild species of section Arachis, determine which diploid species contributed the two genomes of this tetraploid crop and that constitute its primary gene pool, and group and genetic diversity in the genus into primary, secondary, and tertiary gene pools for priorities in selecting species in plant breeding programs.

Approach: DNA will be extracted from leaves of 180 collections of cultivated peanuts and will species. Chloroplast DNA and nuclear ribosomal spacer region will be analyzed for variation in nucleotide sequences and structural organization using ristriction endonucleases and hybridization with known radioactive probes. The data will provide measures of genetic distances and diversities.

CM 25 OTHER OILSEED AND OILSEED CROPS

25.001 CRISO002609
PLANT GERMPLASM INTRODUCTION, INCREASE,
EVALUATION, DOCUMENTATION, MAINTENANCE, AND
DISTRIBUTION

UAIN S K; Agronomy & Range Science; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-ARS-2244-RRProject Type: HATCH Agency ID: CSRS Period: O1 OCT 85 to 30 SEP 90

Objectives: Exploration and Introduction; Increase, Maintenance and Distribution of Plant Germplasm; Germplasm Evaluation and Documentation; Germplasm Resources Information Network (GRIN).

Approach: Continued research on the genetic resources in meadowfoam (Limnanthes spp.) and grain amaranths (Amaranthus spp.) will involve mapping of genetic markers, descriptions of breeding structure and quantitative genetic variation, and agronomic evaluation. Conservation programs in situ as well as ex situ will be developed in cooperation with the California Genetic Resources Program.

Progress: 88/01 to 88/12. Ten populations of rose clover (Trifolium hirtum) were studied in detail for genetic variation using morphological and electrophoretic markers and for life history variation using marked transects in the field. Quantitative genetic analysis involved a total of 300 individual plant families. Outcrossing rates were estimated in five populations using electrophoretic markers. Further experiments on response to artificial selection have been started in Fall 1988. In addition, about 120 accessions were received from the Australian gene bank in order to survey the genetic variation and to establish the source of Californian introductions. Crosses will be made among selected families this Spring in order to study additional genetic markers and to begin gene mapping in rose clover. This work will help initiate an accelerated genetic improvement project on a highly valued range legume.

Publications: 88/01 to 88/12
 No publications reported this period.

25.002 CRISO033893 EXPRESSION OF EMBRYO-SPECIFIC GENES DURING IN VITRO EMBRYOGENESIS IN BRASSICA

CROUCH M C; Indiana University Foundation; Box 1847, Bloomington, **INDIANA** 47402.
Proj. No.: 8200500 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 82 to 28 FEB 86

Objectives: Proj. No. 8200500. We propose to study the expression of embryo-specific genes during the early stages of embryogenesis in Brassica napus L. Early biochemical events have been practically impossible to study in normal embryos of flowering plants because of inaccessibility of the embryo. Therefore, we propose to study adventive embryos that form in culture, unencumbered by endosperm and seed coat tissues, as a model for in vivo development. Using cloned cDNA probes, we plan

to measure stroage protein mRNA levels during in vitro embryogenesis to determine how early and to what extent the embryo program is expressed. From this analysis we hope to learn about the relative importance of quantitative vs qualitative control of gene expression during plant embryo development. Our longterm goal is to understand what initiates and maintains the embryo program.

Approach: The development of a tissue culture system to study early embryo-specific gene expression is a necessary step towards this goal, and is the main objective of our proposal.

Our goal was to Progress: 82/10 to 84/09. develop an embryogenic tissue culture system for Brassica napus to study initiation of storage protein gene expression. We planned to use adventive embryogenesis as a model for in situ development since zygotic embryos are inaccessible early in development. First, we characterized cDNAs for the two major storage proteins, napin and cruciferin. Napin cDNA was sequenced and we showed that the protein is sythesized as a precursor and processed in an interesting way (Crouch et al., 1983). Cruciferin cDNA was also sequenced and compared with a related protein, legmin from peas (Simon and Crouch, in preparation). The comparison identified variable regions which may be good sites for future attempts to insert amino acids necessary for better nutritional qualities of the protein. We then used the cDNAs as probes to study levels of storage protein gene expression in somatic embryos. Attempts to initiate somatic embryos in suspension cultures were unsuccessful. However, we were able to obtain secondary embryogenesis from zygotic embroys cultured between 27 and 32 days after fertilization (Finkelstein and Crouch, 1984; Simon and Crouch, in preparation). These secondary embryos accumulated storage protein mRNAs to levels characteristic of the stage of the primary embryos, then proliferated embryos on their own surfaces: we established a permanently embryogenic tissue culture system which cycled between 0- and 30-day embryo stages.

Publications: 82/10 to 84/09

FINKELSTEIN, R.R. and CROUCH, M.L. 1984. Precociously germinating rapeseed embryos retain characteristics of embryogeny. Planta 162:125-131.

CROUCH, M.L., TENBARGE, K.M., SIMON, A.E. and FERL, R. 1983. cDNA clones for Brassica napus seed storage proteins: evidence from nucleotide sequence analysis that both subunits of napin are cleaved from a precursor polypeptide.

CROUCH, M.L., TENBARGE, K., SIMON, A., FINKELSTEIN, R., SCOFIELD, S., and SOLBERG, L. 1984. Storage protein mRNA levels can be regulated by abscisic acid in Brassica embryos. Im "Molecular Form nnd Function of the Plant CROUCH, M.L. 1983. The use of macromolecular markers to study plant embryo development in vitro. In vitro. 19:238.

25.003* CRISO136273
GENETIC ENGINEERING OF OILSEED SPECIES TO
IMPROVE OIL QUALITY

WURTELE E S; NIKOLAU B J; HAMMOND E G; Food Technology; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: IOWO2893 Project Type: HATCH Agency ID: CSRS Period: 11 OCT 88 to 30 SEP 93

Objectives: Determine biochemical and molecular factors regulating triacylglycerol biosynthesis in developing oilseed embryos and characterize associated genes. Characterize DNA regulatory sequences which direct gene expression specifically during embryo development and test these in transgenic plants.

Approach: Biochemical and molecular studies will be conducted to determine key regulatory steps in the biosynthesis of triacylglycerol. As regulatory enzymes are identified, the genes coding for these enzymes will be cloned, characterized, and appropriate gene constructions will be genetically engineered into oilseed species. The resulting transgenic plants will be tested for altered lipid production. DNA regulatory sequences from genes which we have already obtained which are developmentally regulated during embryo development will be characterized and tested for their ability to direct gene expression in transgenic plants. Such DNA sequences may be used to direct the expression of genes isolated in approach 1.

Progress: 88/10 to 88/12. The laboratory was set up to begin work. To study the biochemical and molecular factors which regulate triacylglycerol biosynthesis in developing oilseed embryos, a model carrot cell and embryo culture system have been established. Aquobacterium strains which can be used for transformation of the carrot cells have been collected. I have begun the characterization of 5 genomic clones which we have shown to be developmentally regulated during embryogenesis.

Publications: 88/10 to 88/12
No publications reported this period.

25.004 CRISO138492 DEVELOPMENT OF NEW AND IMPROVED CROPS FOR WATER CONSERVATION IN ARID LANDS

KNAPP S J; Crop Science; Oregon State
University, Corvallis, **OREGON** 97331.
Proj. No.: OREOO461 Project Type: HATCH
Agency ID: CSRS Period: O1 OCT 88 to 30 SEP 91

Objectives: To improve by breeding and for release, cultivars and germplasm which have superior potential for growth under minimal irrigation or dryland conditions.

Approach: Our research is aimed at increasing knowledge about the genetics of various Cuphea species with seed oils rich in medium-chain fatty acids, and, ultimately, to domesticate a Cuphea species. We are investigating species adapted to the irrigated farming systems of the arid intermountain west. In particular, we are:

Inducing and analyzing genetic variation for fatty acid percentage, fruit morphology, and other traits through the use of conventional chemical mutagenesis and tissue and cell culture (somaclonal variation). Constructing a saturated allozyme and restriction fragment length polymorphism linkage map in C. lanceolata. Investigating the mating and breeding behavior of various Cuphea species. Investigating transformation systems in C. viscosissima. Investigating the cytogenetics of C. viscosissima and C. lanceolata.

25.005 CRISO138452 EVALUATION OF GENETIC VARIABILITY IN ECHINACEA

REESE R N; KAHLER A L; LARSON G E; Biology; S Dakota State University, Brookings, **SOUTH DAKOTA** 57007.

Proj. No.: SD00029-H Project Type: HATCH Agency ID: CSRS Period: 15 MAY 89 to 30 SEP 91

Objectives: Sample representative populations of Echinacea angustifolia to provide materials for isozyme and RFLP polymorphism evaluations of genetic variability within and between native populations, and catalogue phenotypic variability within the species; Conduct cytogenetic analysis of plants from representative populations to determine ploidy levels; develop protocols for isozyme analysis, and observe isozyme polymorphisms and the patterns of distribution of allozyme polymorphisms among and between E. angustifolia samples from across entire geographic range of the species; construct a genomic DNA library and begin isolation of polymorphic markers for RFLP analysis of genetic variability in E. angustifolia.

Approach: Collect seed heads of E. angustifolia along a longitudinal transect and plant out seeds in a breeding nursery; determine ploidy levels using acetocarmine stained root tips and/or microspores; using previously described isozyme systems from sunflower, find polymorphic loci and correlate to quantitative traits; have a genomic library for Echinacea constructed and screen DNA digests to find probes which mark polymorphic loci, using well established procedures.

25.006 CRISO132513 LIPID BIOSYNTHESIS IN LEAVES AND SEEDS

BROWSE J A; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO0792 Project Type: HATCH Agency ID: CSRS Period: 01 SEP 87 to 31 AUG 90

Objectives: To characterize the biochemical defect in plant mutants with altered leaf fatty acid composition and to relate these changes in membrane composition to plant function. To screen Arabidopsis mutants with altered seed

fatty acid composition to determine the limits of mutation breeding in changing the composition of commercial vegetable oils. To isolate the gene encoding the plant phospholipid transfer protein and use it to investigate the role of this protein in lipid metabolism.

Approach: Lipid analysis and radiotracer technique will be used to determine metabolic lesions in mutagenized plants, and the influence of such mutations on photosynthesis will be determined by physiological and biophysical measurements. Chromatographic methods will be used to screen for mutants with alterations in seed fatty acid composition. To determine the function of phospholipid transfer protein, the gene will be isolated and sequenced and the antisense message expressed in transgenic plants.

Progress: 88/01 to 88/12. We have been studying a series of Arabidopsis mutants which have specific alterations in the fatty aicd composition of their leaf lipids. These mutants have helped us to understand the biochemistry of lipid metabolism and the role of membrane lipids in the organization and functioning of plant cells and organelles. Many of the mutants are deficient in one of the desaturases which introduces double bonds into the fatty acid chains of membrane glycerolipids (refs. 4,6). Analysis of a mutant which lacks the chloroplast 16:1/18:1 desaturase (ref. 6) suggested that this desaturase resides mainly in the hydrophobic layer of the membrane with little or no interaction with the hydrophilic lipid headgroups at the membrane surface. Although all the mutant plants grow normally, the changes in the thylakoid membrane composition do have effects on photosynthesis. These include changes in the thermal tolerance of the photoreactions and the balance of activity between photosystems I and II. We have recently completed the characterization of a mutant which lacks activity for chloroplast glycerol-3-phosphate acyltransferase (ref. 1). As a result, one of the two pathways of lipid synthesis is blocked in this mutant. Surprisingly, the defect has a relatively slight effect on the lipid composition of cell membranes because operation of the second lipid synthesis pathway is altered to compensate for the mutation.

Publications: 88/01 to 88/12 KUNST, L., BROWSE, L. and SOMERVILLE, C.R. 1988. Altered Regulation of Lipid Biosynthesis in a Mutant of Arabidopsis Deficient in Chloroplast Glycerol Phosphate Acyltransferase Activity. Proc. Natl. Acad. Sci. 85:4143-4147. BROWSE, J., SOMERVILLE, C.R. and SLACK, C.R. 1988. Changes in Lipid Composition During Protoplast Isolation. Plant Sci. 56:15-20. ZHANG, H., SCHOLL, R., BROWSE, J. and SOMERVILLE, C. 1988. Double Stranded DNA Sequencing as a Choice for DNA Sequencing. Nucl. Acids Res. 216:1220. BROWSE, J., KUNST, L., HUGLY, S. and SOMERVILLE, C. 1988. Modifications to the Two Pathway Schemes of Lipid Metabolism Based on Studies of Arabidopsis Mutants. Proc. 8th International Symposium on the

Biological Role of Plant Lipids.
WU, C.H., CASPAR, T., BROWSE, J., LINDQUIST,
S. and SOMERVILLE, C. 1988.
Characterization of an HSP70 Cognate Gene
Family in Arabidopsis. Plant Physiol.
88:731-740.

BROWSE, J., KUNST, L., ANDERSON, S. and HUGLY, S. 1989. A Mutant of Arabidopsis Deficient in the Chloroplast 16:1/18:1 Desaturase. Plant Physiol. 90: (in press).

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26.001* CRISO011779
PLASMIDS IN PLANT PATHOGENIC BACTERIA

SHAW P D; Plant Pathology; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.

Proj. No.: ILLU-68-0373 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To characterize genetic determinants for phage reception, transfer, and mobilization on plasmid pBPW1 from Pseudomonas syringae pv. tabaci BR2; to characterize the genetic determinant of an outer membrane protein located on that same plasmid; to assess the role of pBPW1 in plant disease; and to characterize pathogenicity genes on the BR2 chromosome.

Approach: Plasmid genetic determinants will be localized by transposon mutagenesis, and plasmid restriction fragments encoding the determinants will be cloned. The cloned fragments will be used to determine gene functions. Pathogenicity genes on the chromosome will be located by transposon mutagenesis, and the cloned mutant genes will be used as probes to isolate functional genes from the BR2 parent. These clones will be characterized structurally and genetically.

Progress: 87/01 to 88/09. Studies have begun on the regulation of expression of pathogenicity genes in plant-pathogenic bacteria. When Tn5 mutants of Pseudomonas syringae pv. tabaci (which were nonpathogenic on tobacco) were inoculated onto leaves of Phaseolus vulgaris (cv. Top Crop), disease symptoms were observed. In some experiments, bacteria reisolated from beans had regained pathogenicity on tobacco, but in other experiments they remained nonpathogenic. In all cases, however, probing restrictions digests of DNA from the reisolated bacteria with labeled Tn5 coupled with growth experiments, indicated that genetic rearrangements had occurred during growth in the bean plants. In one experiment, the transposon (and probably flanking sequences) had been deleted. Our results indicated that the rearrangements were induced by some plant factors. In preliminary experiments to test this, Tn3-HoHol mutants of a 7.2 kb fragment that restores pathogenicity to one of the mutants have been constructed. and expression of a promotorless lacZ gene in the transposon is being tested. Similarly, Tn3-HoHo1 mutants of a 10 kb HindIII fragment that restores pathogenicity to a nonpathogenic mutant of Xanthomonas campestris pv. glycines have also been constructed. Cells containing plasmids with insertions of the transposon in both possible orientations at sites known to contain the pathogenicity genes, fail to produce (beta)-galactosidase in culture. These results indicate that expression of the pathogenicity gene(s) might be inducible by plant factors.

Publications: 87/01 to 88/09
No publications reported this period.

26.002* CRISO089981
CELLULAR AND MOLECULAR GENETICS FOR CROP
IMPROVEMENT

SHAW P D; Plant Pathology; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.

Proj. No.: ILLU-68-0325 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 82 to 30 SEP 88

Objectives: Identification and Characterization of Agriculturally Important Genetic Systems.

Approach: Restriction endonuclease maps will be prepared of the plasmids. These maps will be used as bases for constructing genetic maps, making use of restriction fragments cloned into appropriate vehicles, and transposon mutagenisis. Clones will be characterized by complementation studies, and genes involved in pathogenicity will be characterized.

Progress: 87/10 to 88/09. Xanthomonas campestris pv. glycines (XCG) causes bacterial pustule on soybeans. A soybean cotyledon assay was developed to assay pathogenicity. Fifteen nonpathogenic mutants of strain 8ra were isolated after treatment of the parent with NTG and examining about 2000 individual colonies. Auxotrophic and pigmentless mutants were also obtained. A genomic library was constructed by partial Sau3A1 digestion of total DNA from 8ra and ligating the fragments into pLAFR3. The library was transferred to XCG mutant NP1, and one transconjugant was able to restore pathogenicity to the mutant. It was also able to complement three of the other 15 mutants. A restriction map was prepared of the cloned 30 kb fragment, and deletion analysis indicated that genes that were capable of restoring pathogenicity to NP1 resided on a ten kb HindIII fragment. This fragment was subcloned and subjected to Tn3-HoHol mutagenesis. The insertion mutants were transferred into NP1, and a region of about 2.5 kb within a 2.7 kb Clal fragment was defined as the region containing the pathogenicity genes(s). A 2.2 kb Clal-BglII fragment was subcloned into pW5A, and conjugated into NP1. It did not restore pathogenicity; thus, a region of about 200-300 bp between the BglII restriction site and a Tn3-HoHol insertion appears to be important for gene function. The 2.7 kb Clal fragment has been cloned into pW5A and transferred into NP1. Transconjugants are being tested for pathogenicity, and the fragment is being sequenced.

Publications: 87/10 to 88/09

HUANG, I., LIM, S. M., and SHAW, P. D.
 (1987). Soybean cotyledon bioassay for
 detecting nonpathogenic mutants of
 Xanthomonas campestris pv. glycines.
 Phytopathology 77:1709.

HUANG, I., LIM, S. M., and SHAW, P. D.
 (1988). Generation and complementation of
 pathogenicity genes of Xanthomonas
 campestris pv. glycines 8ra. J. Cellular
 Biochem. 12C:252.

CRISO141190 ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS

SCHAEFFER G W; Beltsville Agr Res Center. Beltsville, **MARYLAND** 20705. Proj. No.: 1275-22000-004-00D

Project Type: INHOUSE

Agency ID: ARS Period: 01 APR 86 to 31 MAR 91

Objectives: Identify, isolate, clone and characterize genes fine structure and functionassociated with elevated lysine levels for plants regenerated from cells resistant to aminoethylcysteine & cells insensitive to inhibitory levels oflysine plus threonine. The first enzyme to be studied in detail is diaminopimelate dehydrogenase whose presence has been demonstrated in rice.

Approach: The structure and function of the gene(s) along the lysine pathway in cereals will be characterized by: a) profiling electrophoretically the seed storage proteins of mutants & the amino acid composition of 30-50 proteins established; b) polyadenylated RNA of variants will be translated in vitro & early polypeptide products separated two dimensionally to estab-lish direct relationships between phenotypes & protein composition, cDNA clones & probes for those clones will be created from amino acid sequences & other methods for the study of gene regulation & temporal expression; c) the fine structure & functional promoters of genes will be defined & sequenced. Additionally work will be extended to determine whether insta- bility induced by tissue culture may be due to the mobilization of DNA elements and to improve plant regeneration from callus and cell suspensions. Beltsville, MD; Rm 127, Bg.O11A; BL-2; 12/85; Scientists & technicians: G. Schaeffer; L. Wenko; F. Sharpe, Jr.; J. Dudley; L. Weaver; L.Baustiloos.

Progress: 88/01 to 88/12. The research has focused on the continued amino acid analyses of single seeds to identify heritable high lysine lines from selfed lines, crosses with original parents and backcrosses to high lysine mutants. Fifth generation seeds are now available and are being prepared for field tests. Tissue culture cell lines of high lysine plants have been established and are being utilized for biochemical isolation of specific proteins and characterization of the lines. Proteins of rice endosperm mutants have been fractionated into solubility classes and the amino acid characteristics determined. The major increase in lysine occurs in the salt soluble globulin fraction. Not only is there a shift in the quantity of individual proteins in the mutant but some types of protein appear to be specifically modified as well in the mutant. Currently unique proteins are being isolated, monitored with 3H-lysine, and will be purified in the weeks ahead. Specific genes will be isolated from these mutant lines. This research will lead to new basic information on the synthesis of lysine in rice and the release of new rice germplasm.

Publications: 88/01 to 88/12

CHOWDHURY, M.K.U., SCHAEFFER, G.W., SMITH, R.L. and MATTHEWS, B.F. 1988. Mole- cular analysis of organelle DNA of diffe rent subspecies of rice and the genomic stability of mtDNA in tissue cultures of rice. Theor. Appl. Genet.76:533-539.
SESEK, S., BOROJEVIC, K. and SCHAEFFER, G.W.

1988. In vitro production of dihpaloids via anther culture in wheat. 7th Internatl. Wheat Genetics Sym- posium, Cambridge University, England, July 13-19, 1988. (Abstract).

SCHAEFFER, G.W. 1988. Segregation for endosperm lysine and protein as well as infertility from crosses of in vitro selected rice. J. Cellular Biochemistry, P roc. 17th Ann. Mtg. UCLA Symp. on Mol. & Cell. Biol., p. 203. (Abstract).

SCHAEFFER, G. 1988. Segregation for endosperm lysine and p rotein as well as infertility from crosses of in vitro selected rice. Proceedings of 22nd Rice Technical Working Group, U. of California, Davis, June 1988. (Abstract).

SCHAEFFER, G.W. 1988. Segregation for increased lysine from crosses of in vitro selected mutants of rice. Proceedings o f 6th Congress of Federation of Euro-pean Societies of Plant Physiology, Split, Yugoslavia, Sept. 1988. (Abstract).

SCHAEFFER, G.W. 1988. Role of microspores and anther culture in advancing technologies. In: Advances in Cell Culture, edited by K. Maramorosch and G. H. Sato. (Book chapter). Academic Press, N.Y.

26.004 CRTS0132808 CELLULAR AND MOLECULAR APPROACHES TO TOBACCO **IMPROVEMENT**

REED S M; Crop Science; North Carolina State University, Raleigh, NORTH CAROLINA 27695. Project Type: HATCH Proj. No.: NCO6036 Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: Determine genetic basis for yield losses in Nicotiana androgenic dihaploids; transfer genes for disease resistance from N. repanda to N. tabacum; develop a detailed chromosome map for N. tabacum.

Approach: DNA quantities in source cultivars and dihaploid populations will be measured using flow cytometry, and correlated with yield data. Interspecific hybridization and gene transfer will be attempted using in vitro fertilization combined with pollen irradiation. Segregating population of N. tabacum will be analyzed for restriction fragment length polymorphisms, which will than be used to construct a detailed map of the N. tabacum chromosomes.

Total nuclear DNA Progress: 88/01 to 88/12. was determined for tobacco cultivars NC95 and Coker 139 and for six androgenic dihaploids from each cultivar. DNA analysis was performed via flow cytometry on isolated nuclei stained with the DNA-specific fluorochrome propidium iodide. Agronomic data also were collected on the DH lines. Results indicated that genotypic effects for nuclear DNA were highly significant. While the DNA increases observed among the NC95 genotypes were not statistically significant, doubled haploids derived from C139 had significantly more DNA per nucleus than C139. Regression analyses of mean nuclear DNA values of C139 genotypes on mean leaf yields resulted in a correlation coefficient of -0.69; this was significant only at the 0.1 probability level. The cytological consequences of this DNA amplification was studied in F1 hybrids between NC95 and a doubled haploid that has 41% more DNA than the NC95. While no cytological abnormalities were observed in either parental line, numerous abnormalities were seen in both somatic and meiotic tissues of the F1 hybrids. Chromosome losses, which appeared to result from spindle errors, were observed in these tissues. In addition to the spindle errors, a quadrivalent with an atypical morphology was observed in meiotic diplotene and metaphase I cells of the hybrid. Further investigations of additional doubled haploid x cultivar lines are currently underway.

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Publications: 88/01 to 88/12

REED, S.M., WERNSMAN, E.A., BURNS, J.A., and KRAMER, M.G. 1988. An evaluation of the use of irradiated pollen for gene transfer in Nicotiana. Plant Sci. 56:155-160.

KRAMER, M.G. and REED, S.M. 1988. An evaluation of maternal nullihaploidy for the production of nullisomics of Nicotiana tabacum L. I. An interspecific hybridization approach. J. Hered. 79:24-27.

JENN, A.E., DAUB, M.E., and REED, S.M. 1988. Limitations of the Su albino gene marker system in fusions between Nicotiana repanda and Nicotiana tabacum. Tob. Sci. 32:77-81.

26.005 CRISO138255 THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIDASE

LAGRIMINI M; Horticulture; Dhio State
University, Wooster, **OHIO** 44691.
Proj. No.: DHOOO502-SS Project Type: STATE
Agency ID: SAES Period: O1 APR 89 to 31 MAR 90

Objectives: Define anionic peroxidase function of tobacco; determine physiological effects to enhance or suppress peroxidase levels; explore feasibility of altering lignin content; record morphological and physiological ramifications of modified lignin content; examine the role of auxin to regulate the expression of anionic peroxidase; and Map regulatory regions within the peroxidase gene by constructing a series of deletions.

Approach: This work will characterize the lignin-forming peroxidase from tobacco. Transgenic plants will be made using the anionic peroxidase cDNA which either overexpress or underexpress this isoenzyme. Regenerated plants will be analyzed for lignin content, wound-healing rate, disease susceptibility, and overall physiology and morphology. The regulatory region of this gene will be joined to the B-glucuronidase reporter gene and transformed into plants. The regulatory region will be subjected to

mutagenesis to determine sequences which confer tissue specific expression and auxin suppression.

26.006* CRISO090986 STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS

RYAN C A; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO1791 Project Type: HATCH Agency ID: CSRS Period: 01 SEP 85 to 31 AUG 90

Objectives: The objectives of this research are to understand the biochemical and molecular biological basis of insect-induced synthesis and accumulation of proteinase inhibitor proteins in plants. The complete chemical nature of the wound signals will be sought and their mechanisms of release, transport, intracellular recognition and gene activation will be studied. The structure and organization of the inhibitor genes will be investigated and the wound-induced promoter regions characterized. These promoters will be employed to improve the natural defenses of important crop plants.

Approach: Modern biochemical, immunological and molecular biological techniques will be employed, including recombinant DNA technology.

Progress: 88/01 to 88/12. Two 5' regions of the wound-inducible potato inhibitor II gene that regulate wound-induction have been identified by deletion analysis in collaboration with Dr. Gyn An. These regions, near 650 bp and 150 bp upstream from the translation initiation codon, were shown by gel retardation assays in this laboratory to be the only regions that bind to specific tomato leaf wound-inducible nuclear proteins. Dne of these trans-factors has been partially purified and has a Mr of 27 kDa. The wound-inducible expression of a fused Inhibitor II-CAT gene in tobacco was shown to be enhanced over 50-fold by sucrose or other metabolizable sugars. This increase is due to transcriptional control, indicating that mRNA synthesis is somehow being regulated by a sucrose-derived molecule. A cDNA coding for wound-inducible trypsin inhibitor in alfalfa leaves (called ATI, a member of the Bowman-Birk inhibitor family) was isolated and characterized. The gene has also been isolated and is currently being characterized. A strongly expressed Inhibitor I gene has been isolated from the DNA of a wild species of tomato, L. peruvianum. This Inhibitor I gene is being introduced into the modern tomato where it is not expressed in fruit. The expression in modern fruit could allow the development of a fruit expression system. Dligosaccharides that induce the accumulation of proteinase inhibitors in plants have been shown to cause the enhanced phosphorylation of plasma membrane proteins from potato and tomato.

- Publications: 88/01 to 88/12
 - CLORE, G.M., GRONEBORN, A.M., NILGES, M. and RYAN, C.A. (1988). The Three-Dimensional Structure of Potato Carboxypeptidase Inhibitor in Solution: A Study Using Nuclear Magnetic Resonance, Distance
 - Geometry and Restrained Molecular Oynamics. RYAN, C.A. and AN, G. (1988). Molecular Biology of Proteinase Inhibitors in Plants.
 - Plant, Cell and Environment 11:345-349.
 RYAN, C.A. (1988). Oligosaccharide Signalling for Proteinase Inhibitors in Plant Leaves.
 In "Advances in Phytochemistry" (Conn. E., ed.) Vol. 22, Plenum Press, NY, pp.
 - 163-180.
 PEARCE, G., LILJEGREN, O. and RYAN, C.A.
 (1988). Proteinase Inhibitors in Fruit of
 the Wild Tomato Species L. peruvianum: A
 possible Mechanism for Plant Protection and
 Seed Dispersal. Planta 175:527-531.
 - AN, G., THORNBURG, R.W., JOHNSON, R., HALL, G. and RYAN, C.A. (1988). A Possible Role for 3' Sequences of the Wound-Inducible Potato Proteinase Inhibitor IIK Gene in Regulating Gene Expression. In "NATO Conference Proceedings".
 - RYAN, C.A. (1988). Proteinase Inhibitor Genes: Strategies for Manipulation to Improve Natural Plant Oefense. BioEssays (in press).
 - GREENBLATT, H.M., RYAN, C.A. and JAMES, M.N.G. (1988). Structure of the Complex of Streptomyces Griseus Proteinase B andPolypeptide Chymotrypsin inhibitor I from Russet Burbank Potato Tubers at 2.1: A Resolution (in Press).

CM 27 SUGAR CROPS

27.001* CRISO137480 BARLEY GENETICS AND PLANT CYTOGENETICS

TSUCHIYA T; HANG A; WANG S; Agronomy; Colorado State University, Fort Collins, COLORADO 80523.

Proj. No.: COLOO625 Project Type: HATCH Agency ID: CSRS Period: 14 FEB 89 to 30 JUN 93

Objectives: The overall objective of this project is basic genetic and cytogenetic studies in various plant species for aiding the progress in genetics and their direct and/or indirect uses in plant breeding programs.

Approach: We use chromosome manipulation approaches in most of the research work. For barley genetics chromosomal mutants, mainly various types of trisomics and many genetic mutants are used to improve genetic linkage maps and study the genetic architecture of barley chromosomes. For other materials, karyotype analysis by conventional and/or Giemsa-banding techniques of chromosome studies will be used.

CRISO142166 CHARACTERIZATION OF THE WORLD COLLECTION OF SUGARCANE AND RELATED GRASSES BY ISOZYME ANALYSIS

KNIGHT R J; WOOD B J; Botany; University of Georgia, Athens, GEORGIA 30602. Proj. No.: 6631-21000-001-01S

Project Type: COOPERATIVE AGREE.

Period: 15 SEP 87 to 15 SEP 88 Agency ID: ARS

Objectives: Use of isozyme analysis to characterize the genetic diversity in the World Collection of sugarcane and related grasses.

Approach: Adapt existing isozyme techniques to sugarcane leaf tissue and determine which enzymes will yield broad and manageable level of data. Once appropriate markers are established with Saccharum spontaneum, sets expected to be similar and sets expected to be dissimilar will be used to verify the ability of the technique to differentiate them. Established techniques will then be used to characterize S. spontaneum and S. officinarum collections (ca. 350 and 800 accessions, respectively), assign them to subgroupings, and analyze for allelic diversity. Groupings based onisozymes will be compared with those based on morphological traits. Isozymedata will also be compared to data obtained by the restriction fragment length polymorphism technique.

Progress: 88/01 to 88/12. Isozyme analysis has been performed with approximately 1500 accessions in the World Collection of Sugarcane and Related Grasses. Of 22 possible enzyme systems tested, only 16 showed sufficient activity to be potentially useful. Ten of these were chosen to be used for the entire study. Three, peroxidase (PER), phosphoglucose isomerase (PGI) and diaphorase (DIA) showed significant levels of polymorphism within and between species. Three others, triosephosphate

isomerase (TPI), malic enzyme (ME) and glutamic oxalic transaminase (GOT) revealed much similarity within the Saccharum accessions. Two enzyme systems, colormetric esterase (CE) and menadione reductase (MNR) produced distinct patterns which are associated with accessions in the genus Erianthus. Further analysis is being conducted to determine if genetic distance measures can be established for these species, which could be useful in predicting breeding behavior and establishing taxonomic relationships.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

27 003 CRISO143178 CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE

MOORE P H; HEINZ D J; TEW T L; Hawaiian Sugar Planter's Assn Experiment Station; Hspa, Po Box 1057, Aiea, HAWAII 96701.

Proj. No.: 5311-22230-001-01S

Project Type: COOPERATIVE AGREE. Period: 01 JUL 88 to 30 JUN 93 Agency ID: ARS

Objectives: Investigate sugarcane physiology and biochemistry at the molecular, cellular, and whole plant levels to identify, characterize, and manipulate genes for improving crop quality and yield.

Approach: Analyze DNA by restriction digested, electrophoresis, southern blotting, and complimentary probes to locate and identify agriculturally important genes. Analyze recombinant RFLP patterns to identify linkage groups and relationship to quantitative trait loci and develop chromosome map. Examinethe structure and function of gene products in metabolism and the development of agriculturally important traits. Develop and refine tech- niques for cell culture, cell fusion, regeneration of plants, and gene transfer. Develop an understanding of the biophysical, physiological, biochemical, and genetic interactions within the sugarcane plant and crop.

Progress: 88/01 to 88/12. Three approaches toward gaining a greater understanding of factors regulating sucrose metabolism and storage were initiated. The biophysical regulation of sucrose cellular transport was investigated through pressure probe experiments on immature and mature stalk storage parenchyma. The young tissue exhibited higher membrane hydraulic conductivity and lower cell wall elasticity than did the mature tissue. Turgor pressures were moderately low in both tissues but rose to high values only in the mature tissue following incubation in water. These results indicate as yet unknown roles for biophysical regulation of sucrose transport. Enzyme assays have been successfully developed for the key enzymes in catabolic and anabolic pathways and fructose 1-6 diphosphate. Experiments are underway to discover treatments of the whole plant which will regulate the activivities of these enzymes in the storage tissue. Enzyme antibody probes and gene probes for the enzyme sucrose synthase have been

CM 27

obtained from Zea Mays and will be used to clarify the role of the enzyme in sugarcane sucrose storage.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

CM 28 MISCELLANEOUS AND NEW CROPS

28.001
MOLECULAR MANIPULATION OF GENES

CRISO132174

SMITH R L; Agronomy; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-AGR-02613 Project Type: STATE Agency ID: SAES Period: 01 JUL 86 to 01 JUL 91

Objectives: To develop a basic molecular understanding of genome organization and gene structure in Napiergrass, sorghum or other promising grasses as biomass crops and to develop the molecular methods and data necessary to identify, modify, and (re)introduce specific genes to these crops that would improve the crop for biomass energy purposes.

Approach: Characterize genome size and organization in Napiergrass and biomass crops; create gene libraries; determine gene structure by isolation and characterization of several specific genes; analyze mechanisms of gene control by DNA sequence analysis and RNA studies of isolated genes; isolate individual chromosomes via pulsed orthogonol field gel electrophoresis in order to create chromosome specific libraries and enhance mapping of genes to and within chromosomes; identify genes for modification and incorporation into Napiergrass (and/or other biomass species); develop vectors and gene constructs for the delivery and expression of modified genes into the protoplasts/cells of biomass plants; introduce modified gene(s).

Progress: 87/10 to 88/09. The Pennisetum purpureum (napiergrass) genome is being characterized using restriction fragment length polymorphism (RFLP) genetic markers to assist genetic improvement and for locating performance and methane conversion enhancing genes. Using probes from a PstI genomic library constructed of P.I. 300086 DNA, we have identified 83 RFLPs. Ten probes can differentiate between the parental napiergrasses, P.I. 300086 and N51 or N75, but only four that can distinguish between N75 and N51. This data indicates that N51 and N75 are much more closely related than is P.I. 300086 to either of them. Those probes are being used to determine the genetic diversity of 20 other napiergrass parentals. To facilitate DNA isolation from the large number of plants necessary for RFLP mapping, three DNA isolation methods used successfully in maize RFLP research were evaluated. None of the methods gave entirely satisfactory results with Pennisetum. DNA isolated by CTAB and isopropanol precipitation methods yielded DNA from some plant genotypes of inadequate quality to restrict. Potassium acetate precipitation of SDS-protein-carbohydrate complexes yielded restrictable DNA from all genotypes, however, it required more work and produced lower DNA yields than the other methods. Plant morpholigical data and tissue for DNA isolation have been collected from two 40-plant progenies of N75 and P.I. 300086 napiergrass X pearl millet crosses in preparation for linkage mapping.

Publications: 87/10 to 88/09
 CHOWDHURY, M.K.U. and SMITH, R.L. 1988.
 Mitochondrial DNA variation in pearl millet
 and related species. Theor. Appl. Genet.
 76:25-32.

28.002 MOLECULAR MANIPULATION OF GENES

CRIS0130650

FERL R J; Vegetable Crops; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-VEC-02613 Project Type: STATE Agency ID: SAES Period: 01 JUL 86 to 30 JUN 91

Objectives: To develop a basic molecular understanding of genome organization and gene structure in Napiergrass, sorghum or other promising grasses as biomass crops and to develop the molecular methods and data necessary to identify, modify, and (re)introduce specific genes to these crops that would improve the crop for biomass energy purposes.

Approach: Characterize genome size and organization in Napiergrass and biomass crops; create gene libraries; determine gene structure by isolation and characterization of several specific genes; analyze mechanisms of gene control by DNA sequence analysis and RNA studies of isolated genes; isolate individual chromosomes via pulsed orthogonol field gel electrophoresis in order to create chromosome specific libraries and enhance mapping of genes to and within chromosomes; identify genes for modification and incorporation into Napiergrass (and/or other biomass species); develop vectors and gene constructs for the delivery and expression of modified genes into the protoplasts/cells of biomass plants; introduce modified gene(s) into biomass plants and analyze the expression of the modified gene(s).

Progress: 87/10 to 88/09. Six genomic clones for sucrose synthase have been isolated from napier grass. Only one of these genes appears to be expressed in napier grass leaf tissue, as well as pearl millet seed. The transcript is smaller than that of the maize counterpart, and the genomic clone apparently lacks sequences found in maize sucrose synthase genes. To identify the progenitors of napier grass, molecular and biochemical analyses were carried out on putative progenitors. A 140 base pair Kpn fragment identifies the A genome of both pearl millet and napier grass. This sequence is extremely highly repetitive, is found in napier grass, and in pearl millet, but is not found in plants which contain only the B genome. The B genome is characterized by a 160 base pair Kpn highly repetitive fragment. This sequence is not found in pearl millet, but is found in a number of related forage grasses. The 140 base pair and the 160 base pair fragments show much sequence identity. Sequencing of representative clones of these fragments reveals a 20 base pair insertion found in the 160 base pair fragment. It would appear that this inertion occurred in the B genome before amplification. At the biochemical level, various isozyme systems were analyzed to determine the origins of the genomes in napier grass. There has been

sufficient sequence diversity through evolution to not allow for the use if isozymes to predictably determine the progenitor of napier grass.

Publications: 87/10 to 88/09

HAUPTMANN, ASHRAF, M., VASIL, V., HANNAH, L.C., VASIL, I.K., FERL, R. 1988. Promoter strength comparisons of maize shrunken 1 and alcohol dehydrogenase 1 and 2 promoters in mono-and dicotyledonous species. Plant Physiol. (in press).

KLEIN, A.S., CLANCY, M., FURTEK, D.B., HANNAH, L.C. and NELSON, O.E. 1988.

HANNAH, L.C., MCCARTY, D.R. 1988. Mature pollen contains transcripts of the constitutive sucrose synthase (Css) gene. Maize Genetics Cooperation Newsletter 62:59.

BOURNIVAL, B.L., VALLEJOS, C.E. CHOUREY, P.S., HANNAH, L.C. 1988. An activity stain for sucrose synthase. Maize Genetics Cooperative Newsletter 62:60.

COBB, B.G., HANNAH, L.C. 1988. Shrunken-1 encoded sucrose synthase is not required for sucrose synthesis in the maize endosperm. Plant Physiology (in press).

BAIER, J.W. 1988. Thesis. University of Florida. DNA sequence differences in wild type alleles of the shrunken gene of maize.

28.003* CRISO138452 EVALUATION OF GENETIC VARIABILITY IN ECHINACEA

REESE R N; KAHLER A L; LARSON G E; Biology; S Dakota State University, Brookings, **SOUTH DAKOTA** 57007.

Proj. No.: SD00029-H Project Type: HATCH Agency ID: CSRS Period: 15 MAY 89 to 30 SEP 91

Objectives: Sample representative populations of Echinacea angustifolia to provide materials for isozyme and RFLP polymorphism evaluations of genetic variability within and between native populations, and catalogue phenotypic variability within the species; Conduct cytogenetic analysis of plants from representative populations to determine ploidy levels; develop protocols for isozyme analysis. and observe isozyme polymorphisms and the patterns of distribution of allozyme polymorphisms among and between E. angustifolia samples from across entire geographic range of the species; construct a genomic DNA library and begin isolation of polymorphic markers for RFLP analysis of genetic variability in E. angustifolia.

Approach: Collect seed heads of E. angustifolia along a longitudinal transect and plant out seeds in a breeding nursery; determine ploidy levels using acetocarmine stained root tips and/or microspores; using previously described isozyme systems from sunflower, find polymorphic loci and correlate to quantitative traits; have a genomic library for Echinacea constructed and screen DNA digests to find probes which mark polymorphic loci, using well established procedures.

28.004 CRISO130204 MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE

BACKHAUS R A; Agriculture; Po Box 388, Weslaco, **TEXAS** 78596.

Proj. No.: ARZR-8601816 Project Type: CRGO Agency ID: CRGO Period: 15 SEP 86 to 30 APR 88

Objectives: PROJ 8601816. Rubber particles purified from guayule contain a single protein. This protein has been purified to homogeneity and is thought to be the enzyme rubber transferase (RuT), the enzyme responsible for rubber formation in all plants. This project will verify if the pure protein is RuT, and a cDNA library will be constructed in an attempt to isolate the gene for rubber formation.

Approach: Antibodies have been raised against the pure protein. These will be used to block the enzymatic incorporation of substrate into rubber in an in-vitro assay. The antibodies will also be used to screen a cDNA library constructed in a pUC or lambda vector, in attempts to isolate the gene. Alternatively, pure protein will be sequenced so that oligonucleotide probes can be used to screen the library.

Progress: 86/09 to 88/04. The goal of this research was to isolate the gene which codes for rubber transferase (RuT), the polymerizing enzyme which causes the last step in the biosynthetic pathway of rubber formation in plants. Prior to this project, our work suggested that RuT was associated with the rubber particles of the guayule plant (Parthenium argentatum). Research supported by this grant was performed to: verify rubber transferase using enzymatic and biochemical techniques to determine whether the rubber particle protein (RPP) purified from guayule was RuT, construct a cDNA library from guayule tissue which are known to produce rubber, screen the cDNA library for clones which contain RuT coding regions. All of these goals were accomplished. It was found that extensively purified rubber particles possess high RuT activity when assayed in the presence of appropriate substrates and cofactors. It was shown that these particles contain only one protein, RPP, which was determined to be RuT by several methods (enzyme kinetics, protein concentration dependence, photolabile-analogue inhibitions). The protein was characterized as a 48.5 kD glycoprotein with a pI of 5.2, which resides within the rubber particle. An amino acid composition and a partial N-terminal sequence was obtained, from which a synthetic oligonucleotide probe was made. A cDNA library was made from nRNA isolated rom guayule bark tissue (a major source of RuT gene expression in this plant) and successfully constructed in lambda gt 10 and gt 11.

Publications: 86/09 to 88/04
BACKHAUS, R.A., BESS, V.H., CHEN, S.F. and CORNISH, K. 1988. Purification and characterization of a protein from enzymatically active rubber particles. Proc. Natl. Acad. Sci. USA. In review.

- BACKHAUS, R.A. and BESS, V.H. 1986. Isolation and characterization of the 50 kD protein from guayule rubber particles. IN: Benedict, C.R. (ed) Biochemistry and Regulation of cis-Polyisoprene in Plants, p. 204-221.
- BACKHAUS, R.A. and BESS, V.H. 1987. Isolating guayule genes for rubber biosynthesis. Guayule Rubber Soc. 7th Ann. Conf., Annapolis, MD.
- CORNISH, K. and BACKHAUS, R.A. 1988. Rubber transferase activity in field grown guayule. Guayule Rubber Soc. 8th Ann. Conf., Mesa, AZ.
- HUANG, D.-S. 1988. Expression and in vitro translation of mRNA for the guayule rubber particle protein. M.S. Thesis. Arizona State Univ. 87 p.
- CHEN, S.-F. 1988. Characterization and immunological analysis of guayule rubber particle protein. M.S. Thesis. Arizona State Univ. 73 p.
- BACKHAUS, R.A. and NAKAYAMA, F.S. 1988. Variation in the molecular weight distribution of rubber from cultivated guayule. Rubber Chem. and Technol. 61:78-85.

28.005* CRISO132513 LIPID BIOSYNTHESIS IN LEAVES AND SEEDS

BROWSE J A; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNP00792 Project Type: HATCH Agency ID: CSRS Period: 01 SEP 87 to 31 AUG 90

Objectives: To characterize the biochemical defect in plant mutants with altered leaf fatty acid composition and to relate these changes in membrane composition to plant function. To screen Arabidopsis mutants with altered seed fatty acid composition to determine the limits of mutation breeding in changing the composition of commercial vegetable oils. To isolate the gene encoding the plant phospholipid transfer protein and use it to investigate the role of this protein in lipid metabolism.

Approach: Lipid analysis and radiotracer technique will be used to determine metabolic lesions in mutagenized plants, and the influence of such mutations on photosynthesis will be determined by physiological and biophysical measurements. Chromatographic methods will be used to screen for mutants with alterations in seed fatty acid composition. To determine the function of phospholipid transfer protein, the gene will be isolated and sequenced and the antisense message expressed in transgenic plants.

Progress: 88/01 to 88/12. We have been studying a series of Arabidopsis mutants which have specific alterations in the fatty aicd composition of their leaf lipids. These mutants have helped us to understand the biochemistry of lipid metabolism and the role of membrane lipids in the organization and functioning of plant cells and organelles. Many of the mutants are deficient in one of the desaturases which

introduces double bonds into the fatty acid chains of membrane glycerolipids (refs. 4,6). Analysis of a mutant which lacks the chloroplast 16:1/18:1 desaturase (ref. 6) suggested that this desaturase resides mainly in the hydrophobic layer of the membrane with little or no interaction with the hydrophilic lipid headgroups at the membrane surface. Although all the mutant plants grow normally, the changes in the thylakoid membrane composition do have effects on photosynthesis. These include changes in the thermal tolerance of the photoreactions and the balance of activity between photosystems I and II. We have recently completed the characterization of a mutant which lacks activity for chloroplast glycerol-3-phosphate acyltransferase (ref. 1). As a result, one of the two pathways of lipid synthesis is blocked in this mutant. Surprisingly, the defect has a relatively slight effect on the lipid composition of cell membranes because operation of the second lipid synthesis pathway is altered to compensate for the mutation.

Publications: 88/01 to 88/12

- KUNST, L., BROWSE, L. and SOMERVILLE, C.R. 1988. Altered Regulation of Lipid Biosynthesis in a Mutant of Arabidopsis Deficient in Chloroplast Glycerol Phosphate Acyltransferase Activity. Proc. Natl. Acad. Sci. 85:4143-4147.
- BROWSE, J., SOMERVILLE, C.R. and SLACK, C.R. 1988. Changes in Lipid Composition During Protoplast Isolation. Plant Sci. 56:15-20.
- ZHANG, H., SCHOLL, R., BROWSE, J. and SOMERVILLE, C. 1988. Double Stranded DNA Sequencing as a Choice for DNA Sequencing. Nucl. Acids Res. 216:1220.
- BROWSE, J., KUNST, L., HUGLY, S. and SOMERVILLE, C. 1988. Modifications to the Two Pathway Schemes of Lipid Metabolism Based on Studies of Arabidopsis Mutants. Proc. 8th International Symposium on the Biological Role of Plant Lipids.
- WU, C.H., CASPAR, T., BROWSE, J., LINDQUIST, S. and SOMERVILLE, C. 1988.
 Characterization of an HSP70 Cognate Gene Family in Arabidopsis. Plant Physiol. 88:731-740.
- BROWSE, J., KUNST, L., ANDERSON, S. and HUGLY, S. 1989. A Mutant of Arabidopsis Deficient in the Chloroplast 16:1/18:1 Desaturase. Plant Physiol. 90: (in press).

CM 30 BEEF CATTLE

30.001* CRISO004941
CONSULTATION AND RESEARCH IN MATHEMATICAL AND
STATISTICAL GENETICS

POLLAK E; Statistics; Iowa State University, Ames, IOWA 50011.

Proj. No.: IOW01448 Project Type: STATE Agency ID: SAES Period: 01 JUL 59 to 01 JAN 99

Objectives: Study of population genetics, with particular reference to balanced polymorphisms maintained by natural selection occurring in human and other species. Consultation on mathematical problems arising from workers in genetics.

Approach: Procedure will consist partly of the examination of theoretical models and will be partly in cooperation with individuals who have collected or are collecting data on genetic populations.

Progress: 88/01 to 88/12. E. Pollak provided assistance to Dr. A. R. Hallauer of the Department of Agronomy, who asked a question concerning covariances between relatives when a population originally has a Hardy-Weinberg structure and successive generations are produced by self fertilization. Let FS(subscript n) be the mean of a full sib family resulting from a cross between two plants that are produced after n generations of selfing. It was verified that the covariance between FS(subscript O) and FS(subscript 4) is equal to the covariance between full sib offspring of individuals of generation O. Assistance was also provided to Mr. Brad Hedges, a student in the Department of Agronomy. He was faced with the problem of calculating what family size is large enough so that, if there are two possible sets of underlying frequencies of K types of offspring of a cross, the probabilities of the two kinds of misclassification are each 0.025. Professor C. P. Cox of the Department of Statistics and E. Pollak collaborated in solving this problem. Previously, the solution was known only if there are two types of offspring.

Publications: 88/01 to 88/12

JUNG, Y. C., ROTHSCHILD, M. F., FLANAGAN, M.
P., POLLAK, E. and WARNER, C. M. Genetic

variability between two breeds based on

restriction fragment length polymorphisms

(RFLPs) of major histocompatability complex

class I genes in the pig.

CM 31 DAIRY CATTLE

31.001* CRISO131898 GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOODS

GLATZ B A; Food Technology; Iowa State University, Ames, IOWA 50011.

Proj. No.: IOWO2827 Project Type: HATCH

Agency ID: CSRS Period: 01 JUL 87 to 30 JUN 92

Objectives: To improve strains of Propionibacterium important in the dairy industry and in propionic acid production. To understand the genetic organization of this and of other Gram-positive organisms. To develop means of gene transfer among these organisms and other Gram-positive organisms. To identify and characterize important genetic determinants in these organisms.

Approach: Plasmids native to propionibacteria will be isolated and characterized. Genes carried on plasmids will be sought, and characterized when found. Conjugations using conjugative plasmids from other Gram-positive organisms or from the propionibacteria will be established. Transformation of DNA into protoplasts or whole cells will be performed. Bacteriophage specific for propionibacteria will be sought. Mutants altered in important traits will be constructed.

Progress: 88/01 to 88/12. The goal of the research is to investigate the genetics of the propionibacteria, which are important industrial organisms. Screening of the culture collection of 119 strains of propionibacteria for the presence of plasmid DNA has been completed. Twenty strains were found to contain plasmids, and at least 10 unique plasmids were identified in these strains. Seven plasmids were partially characterized by restriction endonuclease analysis, and restriction maps were constructed for four of these. Hybridization studies were conducted to determine the relationships among the seven plasmids that were partially characterized. Five of these plasmids were cured from their respective strains by chemical treatment, and all plasmidcarrying strains and cured derivatives were checked for antibiotic resistances, carbohydrate fermentations, and bacteriocine production. Three plasmid-associated traits have been identified: lactose fermentation and a possible cell-surface component on one plasmid, and cell clumping on another plasmid. The culture collection has been screened for the presence of temperate and/or inducible bacteriophage and bacteriocin production, and samples of rumen fluid, Swiss cheese whey, silage, and lake water have been tested for the presence of lytic phage or other inhibitory components. One strain, recently isolated from Swiss cheese, appears to contain a defective bacteriophage.

Publications: 88/01 to 88/12 GLATZ, B.A. and ANDERSON, K.I. (1988). Isolation and characterization of mutants of Propionibacterium strains. J. Dairy Sci. 71: 1769-1776.

31.002* CRISO004941 CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS

POLLAK E; Statistics; Iowa State University,

Ames, IOWA 50011.

Proj. No.: IOW01448 Project Type: STATE Agency ID: SAES Period: 01 JUL 59 to 01 JAN 99

Objectives: Study of population genetics, with particular reference to balanced polymorphisms maintained by natural selection occurring in human and other species. Consultation on mathematical problems arising from workers in genetics.

Approach: Procedure will consist partly of the examination of theoretical models and will be partly in cooperation with individuals who have collected or are collecting data on genetic populations.

Progress: 88/01 to 88/12. E. Pollak provided assistance to Dr. A. R. Hallauer of the Department of Agronomy, who asked a question concerning covariances between relatives when a population originally has a Hardy-Weinberg structure and successive generations are produced by self fertilization. Let FS(subscript n) be the mean of a full sib family resulting from a cross between two plants that are produced after n generations of selfing. It was verified that the covariance between FS(subscript 0) and FS(subscript 4) is equal to the covariance between full sib offspring of individuals of generation O. Assistance was also provided to Mr. Brad Hedges, a student in the Department of Agronomy. He was faced with the problem of calculating what family size is large enough so that, if there are two possible sets of underlying frequencies of K types of offspring of a cross, the probabilities of the two kinds of misclassification are each 0.025. Professor C. P. Cox of the Department of Statistics and E. Pollak collaborated in solving this problem. Previously, the solution was known only if there are two types of offspring.

Publications: 88/01 to 88/12 JUNG, Y. C., ROTHSCHILD, M. F., FLANAGAN, M. P., POLLAK, E. and WARNER, C. M. Genetic variability between two breeds based on restriction fragment length polymorphisms (RFLPs) of major histocompatability complex class I genes in the pig.

CM 35 BEES AND HONEY AND OTHER POLLINATING INSECTS

35.001* CRISO133059
FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR
IDENTIFICATION AND CONTROL OF INSECTS

JOHNSON J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6916 Project Type: HATCH Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

Objectives: 1) Develop flow cytometry and in particular total nuclear DNA content determination as a cytotaxonomic tool, for the recognition of host races, biotypes, and crytic species of insects. 2) Catalogue the nuclear DNA content of insects of economic interest. 3) Develop multiparameter DNA flow cytometry using dyes which are specific for total DNA, adenine and thymine rich repetitive DNA, and guanine and cytosine rich repetitive DNA. 4) Demonstrate the developmental pattern and mode of inheritance of DNA content variation for both euchromatic (unique) and heterochromatic (repetitive) insect DNA sequences. 5) Develop methodology to sort insect nuclei and chromosomes as an aid to gene cloning in insects.

Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

Progress: 88/01 to 88/12. We have now had one year of work with this unique new methodology for the study of heritable variation in insects. We have measured the DNA content of over 1500 individual fire ants including 3 native and two imported species. In addition, we have measured the DNA content of over 200 honey bees, nearly 300 boll weevils, plus smaller number of individuals from 10 other species. The results have been unexpected and exciting. In particular we find: DNA content is a diagnostic tool that aids in identification of species, populations and types. To date, very significant and predictable DNA content differences have been found between 5 fire ants species, between 4 species of boll weevil, and between the Africanized form of the honey bee, the European honey bee and the F-1 hybrid between the two forms. DNA content variation exists within and among some populations. The most extreme variation we have observed occurs in a fire ant population in Walton Co., Georgia, were 129 of 515 males, females, and workers show 3N or triploid DNA amounts. The remainder are diploid or haploid as expected. The smallest significant variation occurs between populations of the boll weevil Anthonomus grandis where DNA content changes are associated with different host plants. DNA change occurs during development. Male fire ants are haploid during early development, but double their DNA before maturity. Boll weevils are largely 2N throughout their life, although in 1/4 to 3/4 of their cells may have 15 to 20 percent additional DNA.

Publications: 88/01 to 88/12

- JOHNSTON, J.S., ELLISON, J.R. and VINSON, S.B. 1989. Flow cytometric determination of insect DNA as an aid to the description, identification, and control of the imported fire ant. Vinson SB McCOWN JL (eds.) Proc. Imported Fire Ant Sympo.
- JOHNSTON, J.S. and ELLISON, J.R. 1989. DNA heterogeniety within and among fire-ants of the genus Solenopsis in southern United States: Evidence from flow cytometry. Submitted to Cytometry.
- JOHNSTON, J.S. and ELLISON, J.R. 1988.
 Triploidy in a polygynous population of the imported fire ant Solenopsis invicta in Walton Co., Georgia. Submitted to Hereditas.
- ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. A method for determination of DNA content of nuclei of insects by flow cytometry. Submitted to Cytometry.
- ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

CM 36 GENERAL PURPOSE SUPPLIES

36.001* CRISO097318
GLYPHOSATE DEGRADATION AND METABOLISM IN
MICROORGANISMS

BRAYMER H D; Microbiology; Louisiana State
University, Baton Rouge, **LOUISIANA** 70803.
Proj. No.: LABO2483 Project Type: HATCH
Agency ID: CSRS Period: O1 JAN 86 to 31 DEC 90

Objectives: To determine the pathway of glyphosate degradation in the Pseudomonas-like microorganism PG2982. Characterize and modify the transport system used by PG2982 to take up glyphosate. To clone the genes involved in glyphosate resistance (AroA) and glyphosate catabolism into E. coli and then into various plants including the soybean.

Approach: The degradation pathway will be determined by identifying labeled products produced from C labeled glyphosate by PG2982 and by using mutants of the organism that are unable to metabolize the herbicide. The transport system will be studied using mutants and purification of the transport proteins. Cloning of the genes will utilize plasmid and phage vectors and an auxotrophic E. coli recipient. The DNA from the E. coli clones will then be subcloned into appropriate plant vectors. These will then be used to introduce the genes into various plants.

Progress: 88/01 to 88/12. Pseudomonas sp. strain PG2982 is highly resistant to the herbicide glyphosate; a potent inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase, the product of the aroA gene of E. coli. We have isolated a plasmid carrying a 2.4-kilobase pair (kb) fragment of DNA from PG2982 capable of increasing the glyphosate resistance of E. coli cells. Preliminary subcloning data suggested that the fragment did not contain the entire gene for glyphosate resistance. In order to isolate a larger fragment of DNA containing the entire gene, a library of large DNA fragments from PG2982 was constructed using a bacteriophage vector. The originally isolated DNA was used to probe this library and a lambda clone carrying the entire gene from PG 2982 was isolated. Subcloning of a 2kb DNA fragment carrying this gene has again resulted in a plasmid capable of increasing glyphosate resistance in E. coli. A protein with a molecular weight of approximately 40,000 is encoded by this plasmid. It is not able to complement the aroA mutation of E. coli strain LC3 and will not hybridize to the e. coli aroA gene. Also, glyphosate cannot be broken down by E. coli cells containing the plasmid. The nucleotide sequence of the gene has revealed the presence of an open reading frame able to encode a protein with a calculated molecular weight of 39,396. Computer analysis has not revealed any significant similarity to other known genes.

Publications: 88/01 to 88/12
FITZGIBBON, J. and BRAYMER, H.D. 1988.
 Phosphate starvation induces uptake of
 glyphosate by Pseudomonas sp. strain
 PG2982. Appl. Environ. Microbiol.
 54:1886-1888.

ROSS, T.K., ACHBERGER, E.C. and BRAYMER, H.D. 1987. Characterization of the Escherichia coli modified cytosine restriction (mcrB) gene. Gene. 61:277-289.

ROSS, T.K., ACHBERGER, E.C. and BRAYMER, H.D. 1989. Identification of a second polypeptide required for McrB restriction of 5-methyl cytosine-containing DNA in Escherichia coli K-12. Molecular and General Genetics. In press.

MURPHY, K.E. and BRAYMER, H.D. 1988.

Molecular cloning and characterization of a genetic region from Serratia marcescens

involved in DNA repair. Molecular

Microbiology. In press.

CM 40 PEOPLE AS WORKERS, CONSUMERS, AND MEMBERS OF SOCIETY

40.001* CRISO133059
FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR
IDENTIFICATION AND CONTROL OF INSECTS

JOHNSON J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6916 Project Type: HATCH Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

Objectives: 1) Develop flow cytometry and in particular total nuclear DNA content determination as a cytotaxonomic tool, for the recognition of host races, biotypes, and crytic species of insects. 2) Catalogue the nuclear DNA content of insects of economic interest. 3) Develop multiparameter DNA flow cytometry using dyes which are specific for total DNA, adenine and thymine rich repetitive DNA, and guanine and cytosine rich repetitive DNA. 4) Demonstrate the developmental pattern and mode of inheritance of DNA content variation for both euchromatic (unique) and heterochromatic (repetitive) insect DNA sequences. 5) Develop methodology to sort insect nuclei and chromosomes as an aid to gene cloning in insects.

Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

Progress: 88/01 to 88/12. We have now had one year of work with this unique new methodology for the study of heritable variation in insects. We have measured the DNA content of over 1500 individual fire ants including 3 native and two imported species. In addition, we have measured the DNA content of over 200 honey bees, nearly 300 boll weevils, plus smaller number of individuals from 10 other species. The results have been unexpected and exciting. In particular we find: DNA content is a diagnostic tool that aids in identification of species, populations and types. To date, very significant and predictable DNA content differences have been found between 5 fire ants species, between 4 species of boll weevil, and between the Africanized form of the honey bee, the European honey bee and the F-1 hybrid between the two forms. DNA content variation exists within and among some populations. The most extreme variation we have observed occurs in a fire ant population in Walton Co., Georgia, were 129 of 515 males, females, and workers show 3N or triploid DNA amounts. The remainder are diploid or haploid as expected. The smallest significant variation occurs between populations of the boll weevil Anthonomus grandis where DNA content changes are associated with different host plants. DNA change occurs during development. Male fire ants are haploid during early development, but double their DNA before maturity. Boll weevils are largely 2N throughout their life, although in 1/4 to 3/4 of their cells may have 15 to 20 percent additional DNA.

Publications: 88/01 to 88/12

- JOHNSTON, J.S., ELLISON, J.R. and VINSON, S.B. 1989. Flow cytometric determination of insect DNA as an aid to the description, identification, and control of the imported fire ant. Vinson SB McCOWN JL (eds.) Proc. Imported Fire Ant Sympo.
- JOHNSTON, J.S. and ELLISON, J.R. 1989. DNA heterogeniety within and among fire-ants of the genus Solenopsis in southern United States: Evidence from flow cytometry. Submitted to Cytometry.
- JOHNSTON, J.S. and ELLISON, J.R. 1988.
 Triploidy in a polygynous population of the imported fire ant Solenopsis invicta in Walton Co., Georgia. Submitted to
- ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. A method for determination of DNA content of nuclei of insects by flow cytometry. Submitted to Cytometry.
- ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

CM 62 SEED RESEARCH

62.001 CRISO140386
ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES
IN THE BARLEY GENOME

CHANDRA G R; MUTHUKRISHNAN S; Agricultural Research Service; Kansas State University, Manhattan, KANSAS 66506.

Proj. No.: 1275-41000-002-01S

Project Type: COOPERATIVE AGREE.

Agency ID: ARS Period: 24 SEP 85 to 30 SEP 86

Objectives: To expand, characterize and maintain the barley genomic library; to study the structure and expression of a-amylase genes using cDNA probes.

Approach: Genomic libraries will be constructed using 10 to 20 kd DNA fragments from nuclear DNA subjected to limited digestion with restriction endonucleases -Sau, 3A, Hae III, Alu I, etc. The DNA fragments will be ligated to the BauH1 arm or the Eco R1 arm of the Vector Charon DNA and packaged to ()particles. Expanded library will be screened with specific cDNA clones to veri-fy whether it contains all members of a gene family. Periodically, the titre of the library will be monitored and amplified to ensure viability. Selected clones will be characterized by using the M13 cloning and sequencing vector system. Specific cDNA probes will be used to elucidate the organization and expression of a-amylase genes in the cereal genome. We will be using modern methods of molecular biology to study transcriptional events leading to the formation of functional mRNAs.

Progress: 85/01 to 85/12. The organization of x-amylase genes in barley genome has been studied using two cDNA clones for these genes which differ in nucleotide sequence. Structural genes for x-amylases are arranged as two subfamilies of genes on barley chromosomes 1 and 6. The genes on the same chromosome share extensive nucleotide sequence identity but differ substantially from those on the other chromosome. The two families of x-amylase genes differ in their response to increasing concentrations of gibberellic acid in the incubation medium and in the kinetics of appearance and/or accumulation of transcripts in aleurone cells. Genomic clones corresponding to the two types of x-amylase genes have been isolated and are being characterized in order to identify the structural basis of the differential hormonal response of the two sets of genes.

Publications: 85/01 to 85/12
NO PUBLICATIONS REPORTED THIS PERIOD.

62.002 CRISO049877
CELLULAR REGULATION OF THE EXPRESSION OF
DORMANT GENES IN CEREAL GRAINS

CHANDRA G R; Seed Research Lab Plant Genetics & Germplsm Inst; Beltsville Agr Res Center, Beltsville, MARYLAND 20705.

Proj. No.: 1280-41000-008-00D

Project Type: INHOUSE

Agency ID: ARS Period: 25 JAN 85 to 25 JAN 88

Objectives: The objectives are 1) to elucidate the hormonal mechanisms that regulate the expression of a-amylase and other dormant genes and 2) determine the structure and organization of the a-amylse genes in the cereal genome.

Approach: cDNA probes will be used to screen the barley genomic library for a-amylasegene sequences. The structure of the a-amylase gene sequences will be de- termined by the M13 cloning and sequencing systems. The gene structure, including the 5' and 3' flanking regions will be characterized, notably forthe state of methylation of the promotor regions. Specific DNA probes willbe used for the insitu hybridization and localization of the a-amylase genes in the chromosomes of the barley-wheat addition lines. We will de- velop and use a hormone responsive protoplast system to elucidate the hor- monal interplay mechanisms that regulate pre-transcriptional activities such as nucleosomal acetylation and phosphorylations reactions associated with the expression of dormant genes in the cereal genome.*.

Progress: 88/01 to 88/12. The DNA sequences of the two a-amylase genes, including promoter regions, are divergent, as are the predicted amino acid sequences of the mature proteins and the N-terminal "leader" peptides. The a-Amy 1 gene contains two introns while the a-Amy 2-gene has three introns. In the coding region, each gene shows 7-10% sequence divergence with respect to the previously characterized cDNA clones of the same gene type. Therefore, differences in nucleotide sequences can account for some of the isozyme variations seen between the sub-families of a-amylases and among members of the same subfamily. Although the nucleotide sequences of the promoter regions of a-Amy and a-Amy 2 genes show little homology, both contain pairs of inverted repeat elements which could constitute regulatory sites. We have discovered that the hormone enhanced "linker-specific" endonuclease and the DNA relaxation activity is associated with the salt soluble chromatin fraction. Furthermore, we found that abscisic acid has no inhibitory or stimulatory effect on these two chromatin enzymes. This is the first report suggesting the involvement of DNA relaxation activity in the hormonal regulation of genome activity of aleurone cells. This research contributes to a better understanding of the hormonal regulation of plant development at the molecular level and focuses on the role of DNA modifying enzymes in gene activation.

Publications: 88/01 to 88/12

KNOX, C., SOUTHAYANON, A.P., CHANDRA, G.R. and MUTHUKRISHNAN, S. 1988. structure and organization of two divergent a-amylase genes from barley. Plant. Mol. Biol. 9:3-17.

MUTHUKRISHNAN, S. and CHANDRA, G.R. 1988.
Regulation of the expression of hydrolase
genes in cereal seeds. Advance in Cereal
Science and Technology, Vol.
IX, p. 129-160. Ed. Y. Pomeyanz.

62.003* CRISO034200
GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN
IN SOYBEAN

POLACCO J C; Biochemistry; University of Missouri, Columbia, MISSOURI 65211.

Proj. No.: MO-OOO19-1 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 84 to 31 AUG 86

Objectives: PROJ 8400505 Recover a soybean seed urease cDNA clone and use it to determine the amount and specificity of urease transcript in cotyledons, leaves and suspension cultures of normal and urease-negative soybeans. Develop the genetic basis of urease-nulls. Explore genetic, nutritional and environmental influences on urease synthesis.

Approach: Identify cloned urease byhybridization to two separate synthetic oligonucleotides. Dot blots, S1 mapping and nuclear run-off transcripts will determine the levels, synthetic rates and type of urease transcripts in various tissues. Analyze revertants, gene dosage effects and allelism with structural gene variants. Screen for new urease nulls. Nutrition and environment will be altered in cultures of cotyledons and suspended cells of normal and urease-negative varieties.

Progress: 84/09 to 86/08. Mutations affecting each of the 2 urease isozymes were recovered in M2 populations of EMS and NMU-treated soybeans. Four mutants allelic with sun (seed urease-null), the lesion responsible for lack of the embryo-specific urease in PI 229324, were recovered. Two have phenotypes indistinguishable from sun, i.e. no detectable embryo-specific urease mRNA or protein. However, two others are leaky. One, n8, appears to accumulate <1% the urease protein of normal seeds. However, the enzyme appears normal by kcat, pH-dependence, heat-stability and aggregation state. The second mutant, n6, makes higher levels of an altered protein: low kcat, increased heat lability, aberrant pH-dependence and aggregation states. Thus sun appears to encode the embryo-specific urease since mutations at sun affect both the level and nature of the urease gene products. We propose that sun and the closely linked Eu locus, which controls urease aggregation state, are actually in the same locus. Urease genomic and cDNA (embryo) has been cloned and partially sequenced. The gene appears to be highly disrupted by introns and so far shows 83% amino acid homology to the sequence determined for isolated Jackbean urease. cDNA and genomic comparisons are underway to assign the genomic clone to one of two urease isozymes, the embryo-specific or the ubiquitous. RFLP's have been found and are being mapped relative to

Publications: 84/09 to 86/08
START, W.G., YU, M., POLACCO, J.C.,
HILDEBRAND, D.F., FREYER, G.A. and
ALTSCHULER, M. Two Soybean Seed
Lipoxygenase Nulls Accumulate Reduced
Levels of Lipoxygenase Transcripts. Plant
Molecular Biology 7:11-23, 1986.
WINKLER, R.G. August 86. Ureide Catabolism in
Nitrogen Fixing Soybean Plants. Ph.D.
Dissertation. University of Missouri.

MEYER-BOTHLING, L.E. December 86. Soybean Mutants Aberrant in Expression of the Urease Isozymes. Masters Thesis. University of Missouri.

62.004* CRISOO81616
GENETIC REGULATION OF THE SOYBEAN UREASE
ISOENZYMES

POLACCO J C: Biochemistry: University of Missouri, Columbia, **MISSOURI** 65211.

Proj. No.: MO-00019 Project Type: HATCH Agency ID: CSRS Period: 01 JUL 85 to 30 JUN 90

Objectives: Since the soybean urease isozymes (ubiquitous and embryo-specific) are differentially regulated and since the ubiquitous form is likely involved in nitrogen fixation we propose to: isolate the structural gene(s) for the embryo-specific and ubiquitous urease isozymes; identify metabolic signals on urease synthesis, induce and select mutations in structural and regulatory genes for each urease, and use cloned urease genes as probes to study the molecular bases of urease regulation and of mutants with altered urease production.

Approach: A urease genomic clone is being used to recover and identify ubiquitous and embryo-specific genes. These will be used to study transcriptional control in cell culture and in both mutant and normal intact plants. Several urease mutants have been recovered.

Progress: 88/01 to 88/12. A fourth urease locus (eu4) has been identified in soybean. A mutation (eu4-aj3) at this locus eliminates urease activity but not antigen in soybean leaves. Roots and callus culture in the eu4-aj3 mutant contain 100 and 40%, respectively, the urease activity of the Williams 82 progenitor. We are testing the hypothesis that eu4 encodes a ubiquitous urease isozyme which is the exclusive species of leaf. These tests include comparison of urease RFLP segregation and the eu4-aj3 trait, and transient expression assay of urease genes of urease in eu4-aj3 leaf propotoplasts. The urease-negative phenotypes of eu2 and eu3-e1 (lacking urease in all vegetative tissue) were shown to be tissue autonomous in graft experiments. The eu1 (embryo-specific urease locus), eu2 and eu3 loci showed no cosegregation with RFLP's revealed by urease clone E15, corroborating deduced amino acid sequence data indicting that E15 does not encode the embryo-specific urease.

Publications: 88/01 to 88/12
WINKLER RG, DG BLEVINS, JC POLACCO, DD
RANDALL. (1988). Ureid catabolism in
nitrogen-fixing legumes. TIBS 13:97-100.
HOLLAND MA, JD GRIFFIN, LE MEYER-BOTHLING, JC
POLACCO. (1987). Developmental genetics of
the soybean urease isozymes. Dev. Genetics
8:375-387.
HOLLAND MA, JD GRIFFIN, JC POLACCO. (1988).

Genetics and molecular biology of two developmentally regulated urease isozymes in soybean. Genome 30:Supp 1, abst. 32.14.37 (16th Intntl Cong Genetics).

GRIFFIN JD, JC POLACCO. (1988). Molecular genetic analysis of a soybean urease genomic clone. Agronomy Abstracts:168 Am Soc of Agronomy, Madison, WI. TORISKY RS. May (1988). Characterization of a urease-like genomic clone from soybean. Masters Thesis - UMC. PARK TK. Aug. (1988). Soybean Embryo Lipoxygenases:Molecular Analysis of Null Mutants and Germination-Specific Species. Masters Thesis - UMC.

62.005 CRISO095959 MITOCHONDRIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS

HANSON, M R; Genetics Development; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-186418 Project Type: HATCH Agency ID: CSRS Period: O5 JUL 85 to 30 SEP 89

Objectives: Characterize the interaction of separate mitochondrial genomes after somatic hybridization; identify the molecular basis of cytoplasmic male sterility (CMS) in plants; identify features of mitochondrial genes important for regulation of expression.

Approach: The function and expression of a mitochondrial DNA region found to be associated with CMS in Petunia will be analyzed and compared to homologous DNA regions in normal fertile plants. Recombinant mitochondrial DNAs present in fertile and CMS somatic hybrid plants will be analyzed. Genes and gene fragments homologous to the sequences identified in the CMS-associated region will be sequenced and RNA transcripts characterized. Sequences important for transcription and translation will be sought by searching for common features of diverse genes and by functional tests in in vitro systems.

Progress: 88/01 to 88/12. A mitochondrial DNA region, previously shown to be genetically associated with cytoplasmic male sterility (CMS) in Petunia, was further characterized by additional sequencing and transcription analysis. The sequence 3' to the S-pcf fused gene was obtained and found to contain two additional open reading frames. Computer homology searches identified the genes as nad3, subunit III of the NADH dehydrogenase complex, and rps12, ribosomal protein 12 of the small mitochondrial ribosomal subunit. These two genes were found to be co-transcribed with the S-pcf gene. A homologous region in the normal fertile line's mitochondrial genome was sequenced and found also to contain nad3 and rps12 genes which were co-transcribed. However, the CMS and fertile mitochondrial genomes differ in that no pcf gene is upstream of nad3 and rps12 in the fertile, and the S-pcf/nad3/rps12 transcripts are much more abundant than the fertile lines' nad3/rps12 transcripts. Improper expression of nad3 and rps12 may play a role in male sterility.

Publications: 88/01 to 88/12
RASMUSSEN, J. and HANSON, M.R. 1988. A NADH
dehydrogenase subunit is co-transcribed
with the abnormal Petunia mitochondrial

gene associated with cytoplasmic male sterility. Mol. Gen. Genet., in press.

62.006* CRISO142956 BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS

MUEHLBAUER F J; Agricultural Research Service, Pullman, WASHINGTON 99164.

Proj. No.: 5348-22230-001-02T

Project Type: INHOUSE

Agency ID: ARS Period: 01 JUL 88 to 30 JUN 93

Objectives: Develop germplasm & cultivars of grain legumes (peas, lentils, and chick peas) that combine pest resistance, stress resistance and quality. Determine mechanisms of imbibitional stress injury to seeds. Identify generic linkages of isozyme and morphological markers with important genes.

Approach: Use known & newly acquired sources (incl. closely related species) of dis- ease & stress resistance for hybridization & selection to develop multiple disease & stress resistant germplasm & cultivars. The germplasm will be developed in agronomically suitable backgrounds. Physiological & biophy- sical properties (e.g., imbibition kinetics, deformation & fracture) of legume seed tissues will be measured. Procedure for evaluating imbibitionalstress injury to germinating seeds are being developed. Segregation patterns (especially homologous linkage groups) of morphological genes & isozyme markers will be used to expand the linkage groups, and select for disease resistance genes. Source of funds is WA & ID Dry Pea & Lentil Commission.

Progress: 88/01 to 88/12. PROJECT WAS NEVER FUNDED - WILL TERMINATED ON WANG.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

62.007* CRISO140267 GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES

MUEHLBAUER F J; SPAETH S C; Grass & Legume Genet & Physiolresearch Unit; Agricultural Research Service, Pullman, WASHINGTON 99164. Proj. No.: 5348-22230-001-00D

Project Type: INHOUSE Agency ID: ARS Period: 14 DEC 84 to 14 DEC 89

Objectives: Develop germplasm and cultivars of grain legumes (peas, lentils, and chick-peas) that combine pest resistance, stress resistance and quality. Determine mechanisms of imbibitional stress injury to seeds. Identify genetic linkages of isozyme and morphological markers with important genes.

Approach: Use known and newly acquired sources (including closely related species) ofdisease and stress resistance for hybridization and selection to develop multiple disease and stress resistant germplasm and cultivars. The germplasm will be developed in agronomically

suitable backgrounds. Physiological and biophysical properties (e.g. imbibition kinetics, deformation and fracture) of legume seed tissues will be measured. Procedures for evaluating imbibitional stress injury to germinating seeds are being developed. Segregation patterns (especially homologous linkage groups) of morphological genes and isozyme markers will be used to expand the linkage groups, and select for disease resistance genes.

Progress: 88/01 to 88/12. Allozyme polymorphisms for 18 loci in Lens were investigated and their monogenic inheritance demonstrated. Linkage relationships among these loci and 4 genes controlling morphological traits revealed 6 linkage groups. Several of these groups appear to be conserved among Lens. Pisum and Cicer. A genetic linkage map of lentil comprising 333 cM was constructed using 20 RFLPs, 8 isozyme and 6 morphological markers. Assuming a genome size of 10 morgans, 50% of the lentil genome could be linked to within 10cM of the 34 markers. Allozyme polymorphisms for 13 loci in Cicer were investigated and monogenic inheritance demonstrated. Linkage analyses showed several small linkage groups. Over 300 germplasm lines of Cicer, mostly C. arietinum, were screened for resistance to Ascochyta blight. About 33 lines exhibited good resistance to the disease. Most recent material originated from USSR. The existence and pathway of cellular pressuredriven extrusion from imbibing seeds was demonstrated using scanning electron microscopy. A method was developed to measure compressive stress which contributes to pressure causing extrusion of intracellular substances. Compressive stress exceeded commonly reported turgor pressure by 2-4 times. An analysis of mechanical stresses in legume seeds during imbibition was begun. A model was developed to describe the coupled uptake by roots of water and solutes and experiments were started to measure parameters needed for the model.

Publications: 88/01 to 88/12 MUEHLBAUER, F.J., N.F. WEEDEN and D.L. HOFFMAN. 1988. Inheritance and linkage relationships of morphological & isozyme loci in lentil (Lens Miller). (Accepted August 1988, Journal of Heredity.). MUEHLBAUER, F.J., W.J. KAISER and Z. KUTLU. 1988. Collection of Lens and Cicer germplasm in Turkey. (Accepted, 1988, Plant Genetic Resources Newsletter.). HAVEY, M.J. and F.J. MUEHLBAUER. 1988. Linkages between restriction fragment length, isozyme, and morphological markers in lentil. (Accepted, July 1988, Theoretical and Applied Genetics.). SUMMERFIELD, R.J., F.J. MUEHLBAUER, and R.W. SHORT. 1988. Controlled environments as an adjunct to field research on lentils (Lens culinaris). (Accepted by Expl. Agric., 1988.). HOFFMAN, D.L., F.J. MUEHLBAUER, G. LADIZINSKY. 1988. Morphological variation in Lens (Leguminosae). Systematic Botany,

13(1):p. 87-96. Over 3 00 germplasm lines

of Cicer, mostly C. arietinum, were

screened for resistance.

MUEHLBAUER, F.J. 1988. Paper presented at the International Workshop on Breeding Dry Beans Phaseolus vulgaris CIAT, Cali, Columbia, November 7-12, 1988.

SPAETH, S.C. 1988. Extrusion of protoplasm and protein bodies through pores in cell walls of pea, bean and faba bean cotyledons during imbibition. Crop Science, Vol. 29, March-April 1989.

62.008* CRISO132513 LIPID BIOSYNTHESIS IN LEAVES AND SEEDS

BROWSE J A; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO0792 Project Type: HATCH Agency ID: CSRS Period: 01 SEP 87 to 31 AUG 90

Objectives: To characterize the biochemical defect in plant mutants with altered leaf fatty acid composition and to relate these changes in membrane composition to plant function. To screen Arabidopsis mutants with altered seed fatty acid composition to determine the limits of mutation breeding in changing the composition of commercial vegetable oils. To isolate the gene encoding the plant phospholipid transfer protein and use it to investigate the role of this protein in lipid metabolism.

Approach: Lipid analysis and radiotracer technique will be used to determine metabolic lesions in mutagenized plants, and the influence of such mutations on photosynthesis will be determined by physiological and biophysical measurements. Chromatographic methods will be used to screen for mutants with alterations in seed fatty acid composition. To determine the function of phospholipid transfer protein, the gene will be isolated and sequenced and the antisense message expressed in transgenic plants.

Progress: 88/01 to 88/12. We have been studying a series of Arabidopsis mutants which have specific alterations in the fatty aicd composition of their leaf lipids. These mutants have helped us to understand the biochemistry of lipid metabolism and the role of membrane lipids in the organization and functioning of plant cells and organelles. Many of the mutants are deficient in one of the desaturases which introduces double bonds into the fatty acid chains of membrane glycerolipids (refs. 4,6). Analysis of a mutant which lacks the chloroplast 16:1/18:1 desaturase (ref. 6) suggested that this desaturase resides mainly in the hydrophobic layer of the membrane with little or no interaction with the hydrophilic lipid headgroups at the membrane surface. Although all the mutant plants grow normally, the changes in the thylakoid membrane composition do have effects on photosynthesis. These include changes in the thermal tolerance of the photoreactions and the balance of activity between photosystems I and II. We have recently completed the characterization of a mutant which lacks activity for chloroplast glycerol-3-phosphate acyltransferase (ref. 1). As a result, one of the two pathways of lipid

synthesis is blocked in this mutant. Surprisingly, the defect has a relatively slight effect on the lipid composition of cell membranes because operation of the second lipid synthesis pathway is altered to compensate for the mutation.

Publications: 88/01 to 88/12

KUNST, L., BROWSE, L. and SOMERVILLE, C.R.

1988. Altered Regulation of Lipid

Biosynthesis in a Mutant of Arabidopsis

Deficient in Chloroplast Glycerol Phosphate

Acyltransferase Activity. Proc. Natl. Acad.
Sci. 85:4143-4147.

BROWSE, J., SOMERVILLE, C.R. and SLACK, C.R. 1988. Changes in Lipid Composition During Protoplast Isolation. Plant Sci. 56:15-20.

ZHANG, H., SCHOLL, R., BROWSE, J. and SOMERVILLE, C. 1988. Double Stranded DNA Sequencing as a Choice for DNA Sequencing. Nucl. Acids Res. 216:1220.

BROWSE, J., KUNST, L., HUGLY, S. and SOMERVILLE, C. 1988. Modifications to the Two Pathway Schemes of Lipid Metabolism Based on Studies of Arabidopsis Mutants. Proc. 8th International Symposium on the Biological Role of Plant Lipids.

WU, C.H., CASPAR, T., BROWSE, J., LINDQUIST, S. and SOMERVILLE, C. 1988.
Characterization of an HSP70 Cognate Gene Family in Arabidopsis. Plant Physiol. 88:731-740.

BROWSE, J., KUNST, L., ANDERSON, S. and HUGLY, S. 1989. A Mutant of Arabidopsis Deficient in the Chloroplast 16:1/18:1 Desaturase. Plant Physiol. 90: (in press).

CM 63 BIOLOGICAL CELL SYSTEMS

63.001* CRISO098702
DEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK
PROTEINS

VIERLING E; Biochemistry; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-136340-H-49-012

Project Type: HATCH

Agency ID: CSRS Period: 01 JUL 86 to 30 SEP 89

Objectives: To determine if plants synthesize additional high molecular weight chloroplast heat shock proteins. If these proteins are found, work will be initiated to obtain corresponding cDNA clones. To determine if chloroplast heat shock proteins are expressed during embryogenesis or germination in the absence of stress. To determine if heat shock proteins can be induced by heat even during embryogenesis and germination. All work is being performed with Pisum sativum cv. "Little Marvel.

Approach: The presence of high molecular weight heat shock proteins will be analyzed by in vitro transport of proteins into isolated chloroplasts. Expression of heat shock proteins will be analyzed by Northern blotting using cloned cDNA probes. When antibodies are available, these will be used to detect proteins.

Progress: 88/01 to 88/12. Results of this research indicate that heat shock proteins function during seed development in the absence of heat stress. Using Pisum sativum as a model system, we have isolated cDNAs encoding HSP70 and four gene families of low molecular weight HSPs. Embryos from seeds developing in the absence of heat stress exhibit significant levels of mRNA for each of these HSPs. We are continuing to characterize the temporal and spatial regulation of HSP mRNA expression during seed development. DNA sequence analysis of one of the low molecular weight HSPs (HSP18) is complete and sequencing of the other clones is in progress. Using HSP70 antibodies, HSP70 proteins can be detected in normal seeds. Several different HSP70 proteins have been detected, one of which is seed specific. Additional cDNA clones for each of the HSP70s have been isolated and are being characterized. To determine if the other HSPs are also synthesized during development, antibodies against each low molecular weight HSP are being produced. HSP18 has been expressed as a fusion protein in E. coli, and is being used for antibody production. To test for similar responses in other plants, cDNAs for heat shock proteins have been isolated from Arabidopsis thaliana. Studies of HSP expression during plant development should yield new insights into HSP function and basic developmental processes.

Publications: 88/01 to 88/12

- VIERLING, E. and DEROCHER, A. (1988). Heat shock mRNAs are expressed during seed development. Plant Physiol. 865:25 (Abstract).
- VIERLING, E., NAGAO, R.T., DEROCHER, A.E., HARRIS, L.M. (1988). A Chloroplast-localized Heat Shock Protein is a Member of a Eukaryotic Superfamily of

Heat Shock Proteins. EMBO J. 7: 575-581.

63.002 CRISO089967
CELLULAR AND MOLECULAR GENETICS FOR CROP
IMPROVEMENT

GURLEY W B; INGRAM L O; INGRAM L O; Microbiology & Cell Science; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-MCS-O2317 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 82 to 30 SEP 88

Objectives: Identification and characterization of agriculturally important genetic systems. Regulation of gene expression and the delivery of genetic material to higher plants and associative microorganisms.

Approach: Agriculturally important genes in the areas of hydrogen uptake in nodulated legumes, alcohol tolerance in microorganisms, and UV radiation damage will be identified and characterized using a variety of genetic and biochemicals methods. The regulation of the transcriptional aspects of gene expression in higher plants will be studied using the T-DNA of Agrobacterium tumefaciens Ti plasmid as model plant genes. The Ti plasmid will also be utilized in the development of a vector for the introduction of genetic material into plant cells.

Progress: 87/10 to 88/09. We have cloned a positive element that resides upstream of the Adh-1 promoter of maize. This TATA-distal promoter element (located from position -410 to -140) contributes 50% of the transcriptional activity of this gene. We are currently analyzing the effects of distance, position, and polarity of orientation on transcriptional activity. We have also constructed a series of vectors that will test the effect of substitution of the 780 gene activator (T-right pTi 15955) on core the promoter of Adh-1. The promoter constructs will be expressed transgenetically in sunflower tumors. S1 nuclease hybrid protection mapping of poly(A) RNA will be used for analysis of mutant transcriptional activity.

Publications: 87/10 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

63.003* CRISO049444
GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS

SCHAEFFER G W; CRISS; MATHEWS; Agricultural Research Service; Beltsville Agr Res Center, Beltsville, MARYLAND 20705.
Proj. No.: 1209-20173-003-00D

Project Type: INHOUSE

Agency ID: ARS Period: 01 OCT 84 to 12 AUG 86

Objectives: Terminate 1209-20173-002, accession 0043396. Start 1209-20173-003 with \$300,000 net to bench funds planned for 1209-20173-002 and \$50,000 from \$4.1 million administrative reduction funds. G. Still is NPS contact. Table 1 - 11F.

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Approach: Twelve on-going projects will receive additional funding to accelerate research on genetic engineering, gene mapping and transfer, hormonal regulation, membrane structure, and other biotechnologies for improved cro p productivity, including control of insects, diseases, and other pests. This high-technology research will improve the fundamental understanding of important agricultural problems and lead to innovative solutions to agricultural problems.

Progress: 84/01 to 84/12. The 160-kilobase chloroplast (ct) DNA of Daucus carota was cloned, and a physical map was constructed. The circular ctDNA map delineates the positions of an inverted repeated region and genes encoding ribosomal RNAs, the large subunit of ribulose biphosphate carboxylase and the 32-kilodalton protein. The ctDNA of a wild species, D. pusillus was also mapped. Although it did show a great deal of variation in restriction endonuclease digestion patterns, the gene positions were found to be conserved. The D. carota 450-kilobase mitochondrial genome was cloned in a bacteriophage lambda vector, and portions of this gene library were partially mapped to the complete circular genome. The ribosomal RNAs and the gene encoding the protontranslocating subunit of the mitochondrial ATPase were localized and mapped. Analysis of flanking regions reveals that these genes are found in only one copy per mitochondrial genome, Changes in gene expression in develoing somatic embryos of D. carota were detected by two-dimensional gel electrophoresis of nascent proteins. Some changes were observed as early as one day following induction of the in vitro developmental sequence by removal of auxin from the culture medium. Putative mutants of D. carota, temperature-sensitive for somatic embryo development, were isolated by a filtration enrichment protocol.

Publications: 84/01 to 84/12 DE BONTE, L.R., MATTHEWS, B.F., and WILSON, K.G. 1984. Variation in plastid and mitochondrial DNAs in the genus Daucus. Amer. J. Bot. 7:932-940. MATTHEWS, B.F., DE BONTE, L.R. 1985. Chloroplast and mitochondrial DNAs of the carrot and its wild relatives. Plant Molec. Biol. Reporter. (In press). MATTHEWS, B.F. and WIDHOLM, J.M. 1985. Organelle DNA compositions and isoenzyme expression in an interspecific hybrid of Daucus. Molec. Gen. Genet. (In press). DIENER, T.O., OWENS, R.A., and CRESS, D.E. 1984. Plant viroids: new diagnostic methods...agriculture. In: Control of Virus Diseases, E. Kurstak and R.G. Marusyk, eds., Dekker, New York, pp. 345-360. OWENS, R.A., KIEFER, M.C., and CRESS, D.E. 1985. Biological activity of cloned ...cDNAs. In: Subviral Pathogens of Plants and Animals, Maramorosch and McKelvey, eds., Academic Press, NY. (In press). HAMMOND, R.W., KIEFER, M.C., CRESS, D.E. and OWENS, R.A. 1984. Probing viroid structure-function...cDNAs. In: Molec. Form and Function of Plant Genome, Plenum, NY. (In press).

63.004* CRISO034200 GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN

POLACCO J C; Biochemistry; University of Missouri, Columbia, **MISSOURI** 65211.

Proj. No.: MO-00019-1 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 84 to 31 AUG 86

Objectives: PROJ 8400505 Recover a soybean seed urease cDNA clone and use it to determine the amount and specificity of urease transcript in cotyledons, leaves and suspension cultures of normal and urease-negative soybeans. Develop the genetic basis of urease-nulls. Explore genetic, nutritional and environmental influences on urease synthesis.

Approach: Identify cloned urease byhybridization to two separate synthetic oligonucleotides. Dot blots, \$1 mapping and nuclear run-off transcripts will determine the levels, synthetic rates and type of urease transcripts in various tissues. Analyze revertants, gene dosage effects and allelism with structural gene variants. Screen for new urease nulls. Nutrition and environment will be altered in cultures of cotyledons and suspended cells of normal and urease-negative varieties.

Progress: 84/09 to 86/08. Mutations affecting each of the 2 urease isozymes were recovered in M2 populations of EMS and NMU-treated soybeans. Four mutants allelic with sun (seed urease-null), the lesion responsible for lack of the embryo-specific urease in PI 229324, were recovered. Two have phenotypes indistinguishable from sun, i.e. no detectable embryo-specific urease mRNA or protein. However, two others are leaky. One, n8, appears to accumulate <1% the urease protein of normal seeds. However, the enzyme appears normal by kcat, pH-dependence, heat-stability and aggregation state. The second mutant, n6, makes higher levels of an altered protein: low kcat, increased heat lability, aberrant pH-dependence and aggregation states. Thus sun appears to encode the embryo-specific urease since mutations at sun affect both the level and nature of the urease gene products. We propose that sun and the closely linked Eu locus, which controls urease aggregation state, are actually in the same locus. Urease genomic and cDNA (embryo) has been cloned and partially sequenced. The gene appears to be highly disrupted by introns and so far shows 83% amino acid homology to the sequence determined for isolated Jackbean urease. cDNA and genomic comparisons are underway to assign the genomic clone to one of two urease isozymes, the embryo-specific or the ubiquitous. RFLP's have been found and are being mapped relative to

Publications: 84/09 to 86/08
START, W.G., YU, M., POLACCO, J.C.,
HILDEBRAND, D.F., FREYER, G.A. and
ALTSCHULER, M. Two Soybean Seed
Lipoxygenase Nulls Accumulate Reduced
Levels of Lipoxygenase Transcripts. Plant
Molecular Biology 7:11-23, 1986.
WINKLER, R.G. August 86. Ureide Catabolism in
Nitrogen Fixing Soybean Plants. Ph.D.
Dissertation. University of Missouri.

MEYER-BOTHLING, L.E. December 86. Soybean Mutants Aberrant in Expression of the Urease Isozymes. Masters Thesis. University of Missouri.

63.005* CRISO081616 GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES

POLACCO J C; Biochemistry; University of Missouri, Columbia, MISSOURI 65211.

Proj. No.: MO-00019 Project Type: HATCH Agency ID: CSRS Period: 01 JUL 85 to 30 JUN 90

Objectives: Since the soybean urease isozymes (ubiquitous and embryo-specific) are differentially regulated and since the ubiquitous form is likely involved in nitrogen fixation we propose to: isolate the structural gene(s) for the embryo-specific and ubiquitous urease isozymes; identify metabolic signals on urease synthesis, induce and select mutations in structural and regulatory genes for each urease, and use cloned urease genes as probes to study the molecular bases of urease regulation and of mutants with altered urease production.

Approach: A urease genomic clone is being used to recover and identify ubiquitous and embryo-specific genes. These will be used to study transcriptional control in cell culture and in both mutant and normal intact plants. Several urease mutants have been recovered.

Progress: 88/01 to 88/12. A fourth urease locus (eu4) has been identified in soybean. A mutation (eu4-aj3) at this locus eliminates urease activity but not antigen in soybean leaves. Roots and callus culture in the eu4-aj3 mutant contain 100 and 40%, respectively, the urease activity of the Williams 82 progenitor. We are testing the hypothesis that eu4 encodes a ubiquitous urease isozyme which is the exclusive species of leaf. These tests include comparison of urease RFLP segregation and the eu4-aj3 trait, and transient expression assay of urease genes of urease in eu4-aj3 leaf propotoplasts. The urease-negative phenotypes of eu2 and eu3-e1 (lacking urease in all vegetative tissue) were shown to be tissue autonomous in graft experiments. The eu1 (embryo-specific urease locus), eu2 and eu3 loci showed no cosegregation with RFLP's revealed by urease clone E15, corroborating deduced amino acid sequence data indicting that E15 does not encode the embryo-specific urease.

Publications: 88/01 to 88/12

WINKLER RG, DG BLEVINS, JC POLACCO, DD

RANDALL. (1988). Ureid catabolism in

nitrogen-fixing legumes. TIBS 13:97-100.

HOLLAND MA, JD GRIFFIN, LE MEYER-BOTHLING, JC

POLACCO. (1987). Developmental genetics of
the soybean urease isozymes. Dev. Genetics
8:375-387.

HOLLAND MA, JD GRIFFIN, JC POLACCO. (1988).

HOLLAND MA, JD GRIFFIN, JC POLACCO. (1988). Genetics and molecular biology of two developmentally regulated urease isozymes in soybean. Genome 30:Supp 1, abst. 32.14.37 (16th Intntl Cong Genetics).

GRIFFIN JD, JC POLACCO. (1988). Molecular genetic analysis of a soybean urease genomic clone. Agronomy Abstracts:168 Am Soc of Agronomy, Madison, WI. TORISKY RS. May (1988). Characterization of a urease-like genomic clone from soybean. Masters Thesis - UMC. PARK TK. Aug. (1988). Soybean Embryo Lipoxygenases:Molecular Analysis of Null Mutants and Germination-Specific Species. Masters Thesis - UMC.

63.006* CRISO090986 STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS

RYAN C A; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO1791 Project Type: HATCH Agency ID: CSRS Period: O1 SEP 85 to 31 AUG 90

Objectives: The objectives of this research are to understand the biochemical and molecular biological basis of insect-induced synthesis and accumulation of proteinase inhibitor proteins in plants. The complete chemical nature of the wound signals will be sought and their mechanisms of release, transport, intracellular recognition and gene activation will be studied. The structure and organization of the inhibitor genes will be investigated and the wound-induced promoter regions characterized. These promoters will be employed to improve the natural defenses of important crop plants.

Approach: Modern biochemical, immunological and molecular biological techniques will be employed, including recombinant DNA technology.

Two 5' regions of Progress: 88/01 to 88/12. the wound-inducible potato inhibitor II gene that regulate wound-induction have been identified by deletion analysis in collaboration with Dr. Gyn An. These regions. near 650 bp and 150 bp upstream from the translation initiation codon, were shown by gel retardation assays in this laboratory to be the only regions that bind to specific tomato leaf wound-inducible nuclear proteins. One of these trans-factors has been partially purified and has a Mr of 27 kDa. The wound-inducible expression of a fused Inhibitor II-CAT gene in tobacco was shown to be enhanced over 50-fold by sucrose or other metabolizable sugars. This increase is due to transcriptional control, indicating that mRNA synthesis is somehow being regulated by a sucrose-derived molecule. A cDNA coding for wound-inducible trypsin inhibitor in alfalfa leaves (called ATI, a member of the Bowman-Birk inhibitor family) was isolated and characterized. The gene has also been isolated and is currently being characterized. A strongly expressed Inhibitor I gene has been isolated from the DNA of a wild species of tomato, L. peruvianum. This Inhibitor I gene is being introduced into the modern tomato where it is not expressed in fruit. The expression in modern fruit could allow the development of a fruit expression system. Oligosaccharides that induce the accumulation of proteinase

inhibitors in plants have been shown to cause the enhanced phosphorylation of plasma membrane proteins from potato and tomato.

Publications: 88/01 to 88/12

CLORE, G.M., GRONEBORN, A.M., NILGES, M. and RYAN, C.A. (1988). The Three-Dimensional Structure of Potato Carboxypeptidase Inhibitor in Solution: A Study Using Nuclear Magnetic Resonance, Distance Geometry and Restrained Molecular Dynamics.

RYAN, C.A. and AN, G. (1988). Molecular Biology of Proteinase Inhibitors in Plants. Plant, Cell and Environment 11:345-349.

- RYAN, C.A. (1988). Oligosaccharide Signalling for Proteinase Inhibitors in Plant Leaves. In "Advances in Phytochemistry" (Conn, E., ed.) Vol. 22, Plenum Press, NY, pp. 163-180.
- PEARCE, G., LILJEGREN, O. and RYAN, C.A. (1988). Proteinase Inhibitors in Fruit of the Wild Tomato Species L. peruvianum: A possible Mechanism for Plant Protection and Seed Dispersal. Planta 175:527-531.
- AN, G., THORNBURG, R.W., JOHNSON, R., HALL, G. and RYAN, C.A. (1988). A Possible Role for 3' Sequences of the Wound-Inducible Potato Proteinase Inhibitor IIK Gene in Regulating Gene Expression. In "NATO Conference Proceedings".
- RYAN, C.A. (1988). Proteinase Inhibitor Genes: Strategies for Manipulation to Improve Natural Plant Defense. BioEssays (in press).
- GREENBLATT, H.M., RYAN, C.A. and JAMES, M.N.G. (1988). Structure of the Complex of Streptomyces Griseus Proteinase B andPolypeptide Chymotrypsin inhibitor I from Russet Burbank Potato Tubers at 2.1: A Resolution (in Press).

63.007* CRISCO96015 THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION

QUAIL P H: Botany; University of Wisconsin, Madison, WISCONSIN 53706.

Proj. No.: WIS-8500295 Project Type: CRG0 Agency IO: CRG0 Period: O1 SEP 85 to 31 AUG 87

Objectives: Proj 8500295. The long-term goal of this research is to understand the molecular mechanism by which phytochrome regulates plant development in response to light. Specific objectives are to define structural properties of the phytochrome molecule potentially responsible for its regulatory function and to

responsible for its regulatory function and understand the molecular basis for the photoreceptor's control of gene expression.

Approach: Sequencing of Avena cDNA clones to deduce the entire amino acid sequence of the polypeptide; sequencing of Avena phytochrome genes and flanking regions as a step toward identifying sequences involved in regulating their transcription; sequencing of phytochrome clones from a dicotyledon in order to identify evolutionarily conserved structural features.

CM 65 INVERTEBRATES

65.001 CRISO136124
MOLECULAR APPROACH TO A GENE CONFERRING
NEMATODE RESISTANCE TO TOMATO

WILLIAMSON V M; Nematology; University of California, Davis, **CALIFORNIA** 95616.

Proj. No.: CA-D*-NEM-5001-CG Project Type: CRGO Agency ID: CRGO Period: O1 JUL 88 to 30 JUN 91

Objectives: PROJ. 8800667. Mi is a dominant locus that confers resistance to root-knot nematodes when present in tomato. Our goal is to clone Mi to increase understanding of the mechanism of resistance conferred by this gene.

Approach: Mi is closely linked to Aps-1, encoding acid phosphatase-1 in tomato. Acid phosphatase-1 will be purified from cell suspension culture and used to produce antibody and obtain peptide sequence for identification of the corresponding cDNA. Using this clone and clones of other linked fragments (RFLPs) as probes, DNA from resistant and susceptible cultivars will be analyzed. Candidate clones of Mi will be obtained by "chromosome walking" techniques. We propose to identify Mi by complementation of function after transformation of susceptible tomato cultivars with candidate clones using Agrobacterium based vectors

Progress: 88/08 to 88/12. We have obtained data that will be of value in our attempt to clone the nematode resistance gene Mi of tomato by chromosome walking from the linked gene Aps-1. Using DNA clones which flank the region of Chromosome VI, which carries Aps-1 and Mi, 7 and 11 genetic map units away, we examined DNA from various tomato cultivars. Southern blot analyses were carried out using DNA from tomato cultivars that differ in the Aps-1 alleles, and in whether they carried Mi. Our results indicate that the size of the region of the tomato genome derived from the wild tomato species L. peruvainum (the source of Mi) varies among cultivars. This region of DNA is quite extensive in some cultivars, where it includes a DNA marker that is 7 map units away from Aps-1. This information will help us to localize the region of DNA encoding Mi for our chromosome walking experiments.

Publications: 88/08 to 88/12
No publications reported this period.

65.002 CRISO130942
IMPROVING THE EFFICACY OF BACULOVIRUS
PESTICIDES BY RECOMBINANT DNA TECHNOLOGY

MILLER L K; Entomology; University of Georgia, Athens, GEORGIA 30602.

Proj. No.: GEO-RC293-110 Project Type: CRG0

Proj. No.: GEO-RC293-110 Project Type: CRGO Agency ID: CRGO Period: O1 OCT 86 to 30 SEP 87

Objectives: PROJECT 8603163. Improve the efficacy of insect baculoviruses as biological pesticides by introducing insect behavior-modifying genes into baculoviruses. Use recombinant DNA technology to genetically construct a baculovirus that expresses a foreign gene which affects insect behavior.

Approach: Construct recombinant baculoviruses that carry a gene encoding an insect-specific neurotoxin and produce large quantities of toxin in infected insect cells. Determine if the recombinant virus is a more effective biological pesticide and if it has an extended host-range. Study possible strategies for reducing recombinant virus persistance in the environment to enhance the ecological safety of the pesticides.

Progress: 86/10 to 87/09. A gene encoding an insect-specific neurotoxin of scorpion venom, the Buthus eupeus insectotoxin I gene (BeIt1), has been synthesized from oligoneucleotides based on the published amino acid sequence of the toxin. The synthetic gene was cloned in E. coli and the sequence of the gene was confirmed by DNA sequencing. The BeIt1 gene was then transferred to an E. coli gene expression vector utilizing the Tac promoter to drive expression. No toxin expression was observed above background endotoxin activity in E. coli. The BeIt1 gene was transferred to a baculovirus expression system utilizing the polyhedrin promoter to drive toxin expression. No toxin activity was observed in recombinant BeIT1 baculovirus-infected cells. Toxin gene expression in infected insect cells was monitored at the protein synthesis level by pulse-labeling proteins with radioactive methionine. Only very low levels of a new 3.7 kilodalton protein was observed in the recombinant virus-infected cells. It is likely that the BeIT1 toxin is highly unstable under the conditions of expression used and if this gene is to be used to enhance baculovirus pesticide efficacy, a means of stabilizing the small polypeptide will need to be developed. The safety of recombinant baculoviruses with respect to mammalian species was also investigated during the course of this work.

Publications: 86/10 to 87/09 CARBONELL, L.F. and MILLER, L.K. 1987. Appl. Environ. Microbioly, 53:1412-1417. Baculovirus interaction with nontarget organisms: A virus-borne reporter gene is not expressed in two mammalian cell lines. CARBONELL, L.F. and MILLER, L.K. 1987. Genetic engineering of viral pesticides: Expression of foreign genes in nonpermissive cells. In "Molecular Strategies for Crop Protection". MILLER, L.K. 1987. Expression of foreign genes in insect cells. In "Biotechnology Advances in Invertebrate Pathology and Cell Culture" (ed. K. Maramorosch). Academic Press, Orlando, Fl.

65.003 CRISO134015 REGULATION OF EXPRESSION OF THE BACULOVIRUS, ACNPV

FRIESEN P D; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843. Proj. No.: IDAOO908 Project Type: HATCH Agency ID: CSRS Period: O1 JAN 88 to O6 JAN 89

Objectives: The long term objective of this proposal is to genetically engineer the baculoviruses for improved efficacy as

biological control agents of insect pests. Our immediate goals are to: determine the organization of several early viral genes for the insertion of foreign genes, locate those DNA sequences responsible for early and regulated expression of such genes, and examine the function of the early genes determing whether they are nonessential and are therefore replaceable. This proposal also examines the nature and mutagenic effects of an insect-derived transposable element which has integrated into the DNA genome of the baculovirus, AcNPV. Transposable elements act to decrease the virus' ability to produce its occluded form thereby reducing viral pesticide effectiveness.

Approach: Viral gene organization and the location of DNA control regions will be analyzed by fusing specific genes to easily assayed reported genes and testing them for proper expression in transient assays and by placing them back into the viral genome. Antibodies raised to trihybrid viral fusion proteins will be used to examine viral gene function. The gene organization and mutagenic effects of the transposable element will be determined by DNA sequence analysis and nucleic acid hybridization techniques.

Progress: 88/01 to 88/12. Progress in our investigation of the molecular mechanisms involved in the regulation of baculovirus gene expression has been the identification of DNA sequences responsible for conferring early and late transcription of the gene encoding a 35,000 - molecular - weight protein (35K) in the HindIII-K genome region of Autographa californica nuclear polyhedrosis virus (AcNPV). Mutagenesis of the 35K gene promoter previously linked to the reporter gene for chloramphenicol acetyl-transferase, indicated that sequences from -155 to -55 relative to the RNA start site (position +I) controlled early transcription while sequences from -55 to -4 controlled late transcription in recombinant viruses. Thus, two distinct regions of the 35K gene promoter are involved in early versus late regulation. Both regions contain sequences found at the promoter of other AcNPV genes with similar regulation and may therefore represent common control sequences. These studies on the nature of AcNPV promoters provide necessary information for the construction of recombinant baculoviruses expressing insecticidal genes for improved biological control of insect pests.

Publications: 88/01 to 88/12
NISSEN, M.S., and FRIESEN, P.D., (1989).
Molecular Analysis of the Transcriptional
Regulatory Region of Early Baculovirus
Gene. J. Virology (in press).

65.004 CRISO083485 ORGANIZATION AND EXPRESSION OF A BACULOVIRUS DNA GENOME

MILLER L K; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843. Proj. No.: IDAOO801 Project Type: HATCH Agency ID: CSRS Period: O1 JUL 84 to 30 JUN 89

Objectives: The long-term objectives of this proposal are to improve the efficacy of viral pesticides by genetic engineering technology and facilitate the commercial production of these viruses. To achieve these objectives, more information conce ning viral gene organization and the regulation of gene expression is required. The immediate goals are, therefore, to locate key viral genes with respect to a physical map of the DNA genome, test an early promoter for controlling early expression of a passenger gene, and define the nature of genes involved in controlling gene expression. Since a handicap in the commercial production of viruses in cell culture was traced to the insertion of mobile genetic elements into the viral DNA.

Approach: Key genes of the baculovirus AcNPV will be mapped with respect to the established physical map of the viral DNA by marker rescue and by cloning cDNAs of early and intermediate viral mRNAs. Detailed knowledge of the nature of one such gene will be obtained; its promoter will be fused to an easily assayable gene and tested for temporal regulation. The position preferences of transposable element insertions will be determined and their effect on viral gene expression will be determined by nucleic acid hybridization techniques.

Progress: 86/01 to 86/12. Research has progressed in several different areas relevant to baculovirus gene orgainzation and expression that are applicable to development of more effective viral pesticides. First, we have synthesized and cloned complementary DNA from 20 different regions of the viral genome. Temporal expression of RNA from each region was examined and all were found to contain overlapping sets of RNA. Many of these overlapping sets of RNA have common 5' or common 3' termini, a common motif in the organization and expression of baculovirus genes. These studies are important since an understanding of the regulation of viral expression is required before the virus can be successfully engineered (via recombinant DNA) as improved pesticides. Secondly, to better understand this regulation, we have analyzed the structure of viral DNA during the viral replication cycle. We found that the viral genome adapts a nucleosomal-like structure typical of DNA undergoing active transcription. Thirdly, we have continued studies on the molecular biology of a transposable element (TED) which inserted into the baculovirus genome causing mutations. This led to the discovery that TED is a member of a newly characterized class of mutagenic elements which resemble the RNA tumor viruses.

Publications: 86/01 to 86/12

WILSON, J. and MILLER, L.K. 1986. Changes in the nucleoprotein complexes of a baculovirus DNA during infection. Virology 151:315-328.

MAINPRIZE, T.M., LEE, K.-J. and MILLER, L.K. 1986. Variation in temporal expression of overlapping baculovirus transcripts. Virus Res. 6:85-89.

FRIESEN, P.D., RICE, W.C., MILLER, D.W. and MILLER, L.K. 1986. Bidirectional transcription from a solo long terminal

CM 65

repeat of the retrotransposon Ted: Symmetrical RNA start sites. Mol. Cell. Biol. 6:1599-1607.

CM 66 MICROORGANISMS

66.001* CRISO131223
MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN
PSEUDOMONAS SYRINGAE PV. GLYCINEA

STASKAWICZ B J; Plant Pathology; University of California, Berkeley, **CALIFORNIA** 94720. Proj. No.: CA-B*-PPA-4786-CG Project Type: CRGO Agency ID: CRGO Period: O1 SEP 86 to 30 SEP 88

Objectives: PROJECT 8600299. The major objectives of this research are to determine the nucleotide sequence of the avirulence genes avrA, avrB and avrC and to study the expression of these genes both in vitro and in planta.

Approach: The determination of the primary nucleotide sequences will allow us to construct precise beta-galactosidase fusions. The fusion proteins will then be used to raise antisera and to analyze the expression of this protein both in vitro and in planta. In addition, we will attempt to localize the avirulence protein in subcellular fractions employing western blot procedures. The ultimate goal of this project is to determine the molecular basis of specificity and the induction of disease resistance in the bacterial blight disease of soybean.

Progress: 86/09 to 88/09. Pseudomonas syringae pv. glycinea is the causal agent of bacterial blight disease of soybean. The expression of disease resistance in the cultivar Harosoy is dependent on the resistance gene Rpgl and the presence of the avirulence gene avrB in Psg RO. In addition, the avirulence gene avrB is induced during the expression of disease resistance, while the bacterium is growing in the host. Experiments have been performed that have characterized the promoter region of avrB. The initiation of transcription has been determined by primer extension and has been identified to be 78 bp upstream from the initiation of translation. Finally, mutations in the hrp gene cluster have been identified that regulate the induction of avrB.

Publications: 86/09 to 88/09

TAMAKI, S., D. KOBAYASHI, B. STASKAWICZ, and N. T. KEEN. (1988). Construction of recombinant genes from avrB and avrC in order to localize regions of plant recognition specificity. Ann. Symp. Plant Path., Univ. of California, Riverside.

TAMAKI, S., D. DAHLBECK, B. STASKAWICZ and N. T. KEEN. (1988). Characterization and expression of two avirulence genes cloned from Pseudomonas syringae pv. glycinea. J. Bact. 170:4846.

66.002* CRISO089967 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT

GURLEY W B; INGRAM L O; INGRAM L O; Microbiology & Cell Science; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-MCS-O2317 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 82 to 30 SEP 88

Objectives: Identification and characterization of agriculturally important genetic systems. Regulation of gene expression and the delivery of genetic material to higher plants and associative microorganisms.

Approach: Agriculturally important genes in the areas of hydrogen uptake in nodulated legumes, alcohol tolerance in microorganisms, and UV radiation damage will be identified and characterized using a variety of genetic and biochemicals methods. The regulation of the transcriptional aspects of gene expression in higher plants will be studied using the T-DNA of Agrobacterium tumefaciens Ti plasmid as model plant genes. The Ti plasmid will also be utilized in the development of a vector for the introduction of genetic material into plant cells.

Progress: 87/10 to 88/09. We have cloned a positive element that resides upstream of the Adh-1 promoter of maize. This TATA-distal promoter element (located from position -410 to -140) contributes 50% of the transcriptional activity of this gene. We are currently analyzing the effects of distance, position, and polarity of orientation on transcriptional activity. We have also constructed a series of vectors that will test the effect of substitution of the 780 gene activator (T-right pTi 15955) on core the promoter of Adh-1. The promoter constructs will be expressed transgenetically in sunflower tumors. \$1 nuclease hybrid protection mapping of poly(A)RNA will be used for analysis of mutant transcriptional activity.

Publications: 87/10 to 88/09 NO PUBLICATIONS REPORTED THIS PERIOD.

66.003* CRISO131156 EFFICIENCY OF NITROGEN FIXATION

DAVIS L C; Biochemistry; Kansas State
University, Manhattan, KANSAS 66506.
Proj. No.: KANOO648 Project Type: STATE
Agency ID: SAES Period: 01 JUL 87 to 30 JUN 90

Objectives: This research has two different specific objectives. First, mutagenesis and DNA sequencing will be used to determine amino acid substitutions that alter the function of the Fe protein of nitrogenase from Klebsiella pneumoniae. Mutations will be produced ex situ (in E. coli) and transferred back into K.pneumoniae to characterize the functional nature of the lesions induced. DNA sequencing will be used to identify the locations within the protein sequence that have been mutated.

Approach: Second, 2 dimensional gel electrophoresis and isoelectric focussing will be used to identify proteins specific to root nodules in Glycine max (soybean cultiva BAY) and G. tomentella, and in an interspecific hybrid. Comparison of the proteins produced during nodulation rhizobial strains specific to each of the parental species will reveal whether there are common switching mechanisms in the hybrid that respond to the infection process per se or whether there are species

specific switches that turn on only genes associated with a particular genome in the infection process.

Progress: 88/01 to 88/12. This research has been focused on efforts to understand the way in which the host plant genome determines the infection process for different strains of rhizobium. Work has been continued on the way in which hybrids of Glycine max x Glycine tomentella respond to different strains of rhizobia including both fast and slow growers. Gel electrophoresis of enzymes and proteins specific to root nodules has been expressed. This work was reported at the 7th international symposium on nitrogen fixation, Cologne, Germany. This year a method was developed for distinguishing between acetylene reduction and endogenous ethylene production. It depends on the use of specific infrared absorption bands of the undeuterated and deuterated acetylenes and ethylenes to distinguish between ethylene derived from deuterated acetylene and ethylene produced from other substrated without deuterium. A paper detailing the procedure is in press. A fast-growing wide host range rhizobium isolated from a prairie legume Amorpha has been further characterized for its host range. This is the first report of isolation of such an organism with the ability to nodulate soybean in North America. Its relationship to other fast-growing rhizobia isolated in China is being determined by DNA restriction mapping.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

CRISO131900 DEFINING AND MAPPING THE GENES OF **CAULIMOVIRUSES**

SHEPHERD R J; Plant Pathology; University of Kentucky, Lexington, KENTUCKY 40506. Proj. No.: KY00872 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 90

Objectives: To study the functions and role in pathogenesis of the genes of cauliflower mosaic virus (CaMV), a small DNA virus of plants.

Approach: The virus genome has been completely sequenced and the specific location of each tentative gene is precisely known and a catalog of restriction sites is known for use in restructuring the DNA. The DNA can be cloned in bacteria and is infectious to plants when cut free of the cloning vector. Hence, with recombinant DNA methods it is easy to manipulate the virus genetically. In addition single virus genes are being inserted in the plant chromosome using the Ti plasmid-Agrobacterium system. Various promoters of different strength will be coupled to CaMV gene V1 and integrated into plant chromosomes to relate magnitude of disease with gene V1 expression. In addition the cytological effects and virus susceptibility of gene V1 transformed plants will be determined by convent anal methods.

Progress: 88/01 to 88/12. An integrative transformation strategy is being used to evaluate the level and character of disease induced by caulimovirus gene VI expression in host plants. We have cone Ti-plasmid-Agrobacterium transformations of gene VI of cauliflower mosaic (CaMV) and figwort mosaic (FMV) viruses in Nicotiana tabacum or Datura innoxia. Transformation with gene VI of either virus induces a chlorosis and mottling-type disease similar in appearance to virus infections. Symptom development is closely associated with high level expression of the gene VI protein (P62). However, in most cases where symptoms develop (CaMV or FMV in N. tabacum or CaMV in D. innoxia) the plant is not a systemic host of the virus in question. For this reason we have developed a procedure for regenerating Nicotiana edwardsonii which is a systemic host for all three viruses (CaMV, FMV and peanut chlorotic streak viruses). Consequently, we are now in a position to determine if gene VI expression will perturb natural systemic hosts to cause disease. Gene VI RNA transcripts are being electroporated into protoplasts followed by treatment of extracts with chemical cross linkers followed by Western blotting analyses for P62 in an effort to discover if this protein becomes closely associated with a host protein in order to carry out its function. This approach has not yielded positive results yet.

Publications: 88/01 to 88/12 SHEPHERD, R.J., GOLDBERG, K-B., KIERNAN, J., GOWDA, S., SCHOELZ, J., YOUNG, M. and RICHINS, R. (1988). Genomic changes during host adaption by caulimoviruses. In Physiology and Biochemistry of Plant-Microbial Interactions. SHEPHERD, R.J. (1988). The biochemistry of DNA plant viruses. In The Biochemistry of Plants, a Comprehensive Treatise. Vol. 6. Proteins and Nucleic Acids. P. Stumpf and E.E. Conn, Eds. Academic Press, pp. 565-618.

CRISO141958 PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAD DISEASES OF FRUIT TREES

CIVEROLO E L; Beltsville Agr Res Center, Beltsville, MARYLAND 20705. Proj. No.: 1275-24000-052-00D

Project Type: INHOUSE

Period: 22 AUG 86 to 21 AUG 91 Agency ID: ARS

Objectives: (1) Study pathogenecity and develop improved disease diagnostic and identification techniques for Xanthomonads; (2) identify genes affecting virulence and host recognition of X. campestris pv. citri and (3) evaluate the role of bacteriophages in the biological control of Xanthomonads causing disease in fruit trees.

Approach: Comparative characterization of phytopathogenic xanthomonads including pathogenicity, serological characteristics, plasmid and genome DNA content, phage sensitivity, fatty acid composition, aminopeptidase activity, and isozyme activity. Develop rapid, specific, sensitive and reliable

1

methods to detect and identify phytopathogenic prokaryote fruit tree pathogens. Develop improved inoculation and bioassay techniques to quantitatively assess pathogenicity and virulence of phytopathogenic bacteria of fruit trees and to quantitatively assess host resistance or susceptibility to infection. Determine the nature of pathogenicity of xanthomonads of fruit trees, including specific recognition phenomena and virulence factors. Identification, cloning and physical characterization of genes affecting virulence of X. c. pv. citri. Study the role of bacteriophages and antagonistic microorganisms in pathogenesis of phytopathogenic bacteria of fruit trees and development of diseases caused by these bacteria.

Progress: 88/01 to 88/12. Continued studies on the characterization of diverse Xanthomonas strains isolated from citrus including Xanthomonas campestris pv. citri (Xcc) and X. campestris (Xc) associated with citrus bacterial canker (CBC) and spot (CBS) diseases, respectively. Additional strains of Xcc from Saudi Arabia were added to the existing collection at BARC. These strains can be differentiated by serology, plasmid DNA content, genomic DNA fingerprinting, restriction fragment length polymorphism (RFLP) and phage typing. Continued evaluation of a detached leaf bioassay further for selective isolation of Xcc and Xc variants, analyses of host-pathogen interactions and evaluation of differential host response(s) of citrus germplasm in vitro. Quantitative and qualitative evaluation of the pathogenicity of Xcc and Xc strains on detached Citrus spp. leaves by probit analyses of inoculum dose-host response in infectivity titration assays was continued. Collectively, this information can be used to differentiate XCC and XC variants. This information will be used by other researchers and by State and Federal action agencies, such as APHIS. Transposon mutagenesis identified pathogenicity mutants of Xcc which will be complemented from library of strain Xcb2.

Publications: 88/01 to 88/12
 REISTACHER, C.N. and CIVEROLO, E.L. 1989.
 Citrus bacterial canker disease of li me
 trees in the Maldive Islands. Plant
 Disease. Accepted 10/5/88.

GRAHAM, J.H., COTTWALD, T. and CIVEROLO, E.L. 1989. Population dynamics and survival of Xanthomonas campestris in soil and simulated citrus nurseries in MD and Argentina. Plant Disease. Accepted 12/21/88.

66.006* CRISCOBB813 GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANDII

BISHOP P E; Microbiology; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.

Proj. No.: NCO5531 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 82 to 30 NOV 83

Objectives: Isolate Nif mutants defective for both the molybdo-enzyme N(2) fixation system and the recently discovered alternative system. Characterize these mutants using both biochemical and genetics techniques. Clone restriction endonuclease-generated fragments of chromosomal DNA containing nif genes. Characterize the cloned nif DNA using restriction, marker resaue and DNA sequence analysis.

Approach: Ethylmethane sulfonate (EMS) and Tn5 mutagenesis procedures will be employed in the isolation of Nif mutant strains of A. vinelandii. The Nif mutant strains will be characterized using high resoulution 2-dimensional gel electrophoeesis, by titration and extracts prepared from mutant cells with purified nitrogenase components, and by genetic mapping. Genes for the conventional molybdo-enzyme and alternative N(2) fixation systems will be cloned into Escherichia coli.

Progress: 85/01 to 85/12. Deletion strains of A. Vinelandii which are missing the structural genes (nifHDK) have been used to provide conclusive evidence for the presence of an alternative Nitrogen fixation system in this organism. An A. vinelandii nifH probe has been used to identify clones containing nifH-like sequences in an EMBL-3 genomic library constructed from a nifHDK deletion strain (CA11). The partial base sequence of one of these nifH genes has been determined. The alternative nitrogenase proteins have been partially purified from strain CA11.6 (carries nifHDK deletion and is tungsten tolerant). Isolation of a transposon mutant containing Tn5 inserted in nifB (required for FeMo-co synthesis) has facilitated the cloning of a 3.6-kb EcoR1 fragment containing the nifB gene. Since this gene is required for functioning of the alternative Nitrogen fixation system, sequencing this gene should provide further insight into the genetics of this system.

Publications: 85/01 to 85/12

- BISHOP, P.E., RIZZO, T.M. AND BOTT, K.F. 1985. Molecular cloning of nif DNA from Azotobacter vinelandii. J. Bacteriol. 162-21-28.
- SMITH, B.E., et al. R. 1985. The iron-molybdenum cofactor of nitrogenase. pp.
- 597-603. In Nitrogen Fixation Research Progress, Martinus Nijhoff Publishers, Boston. 731 pp.
- KENNEDY, C., et al. 1985 Genetic and physical studies of nif and ntr genes in Azotobacter chroococcum and A. vinelandii. pp. 469-476. In Nitrogen Fixation Research Progress, Martinus Nijhoff Boston. 731 pp.
- BISHOP, P.E. AND EADY, R.R. 1985. Nitrogen fixation by a nifHDK deletion strain of Azotobacter vinelandii. p. 622. Nitrogen Fixation Research Progress, Martinus Nijhoff, Boston. 731 pp.

66.007* CRISO091368
GENETICS OF NITROGEN FIXATION IN AZOTOBACTER
VINELANDII

BISHOP P E; Microbiology; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO5531 Project Type: STATE Agency ID: SAES Period: O1 OCT 82 to 30 SEP 87

Objectives: Isolate Nif- mutants defective for both the molybdo-enzyme N2 fixation system and the recently discovered alternative system. Characterize these mutants using both biochemical and genetics techniques. Clone restriction endonuclease-generated fragments of chromosomal DNA containing nif genes. Characterize the cloned nif DNA using restriction, marker rescue and DNA sequence analysis.

Approach: Ethylmethane sulfonate (EMS) and Tn5 mutagenesis procedures will be employed in the isolation of Nif mutant strains of A. vinelandii. The Nif- mutant strains will be characterized using high resolution 2-dimensional gel electrophoresis, by titration of extracts prepared from mutant cells with purified nitrogenase components, and by genetic mapping. Genes for the conventional molybdo-enzyme and alternative N2 fixation systems will be cloned into Escherichia coli.

Progress: 82/10 to 87/09. Deletion strains of Azotobacter vinelandii lacking the structural genes for conventional Mo-containing nitrogenase were shown to fix nitrogen under Mo-deficient conditions. This provided definitive proof for the existence of an alternative nitrogen fixation system in this diazotroph. An alternative nitrogenase was purified from one of the deletion strains. This nitrogenase is expressed only in the absence of V and Mo and does not contain either element. Genes thought to encode this nitrogenase have been cloned and partially sequenced. Transcripts from these genes are repressed by both V and Mo. Mutants (Tn5-mediated) which are unable to express the alternative nitrogen fixation system have been isolated and partially characterized. The wild-type allele for one of these mutations has been cloned on a 3.8-Kbp EcoRl fragment of DNA. This entire fragment has been sequenced and shown to contain the nifB-nifQ region of the A vinelandii genome. Northern blot analysis using probes from this region indicate that it is a nif operon which is expressed in the presence or absence of Mo or V. However, the operon is repressed by ammonia. Insertion mutations in this operon indicate that nifB is required for nitrogen fixation under all conditions but nifQ is not required for normal functioning of the alternative nitrogenase system.

Publications: 82/10 to 87/09 NO PUBLICATIONS REPORTED THIS PERIOD. 66.008* CRISO132499
CHARACTERIZATION OF AN ALTERNATIVE NITROGEN
FIXATION SYSTEM IN AZOTOBACTER VINELANDII

BISHOP P E; Microbiology; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO5650 Project Type: STATE Agency ID: SAES Period: O1 OCT 87 to 30 SEP 92

Objectives: Sequence both nifH-homologous and flanking DNA sequences which have been cloned from the nifHDK deletion strain CA11 and analyze the sequences for ORF's and promoter regions. Sequence cloned DNA's which contain wild-type alleles for Tn5 mutations that confer a Nif-phenotype under Mo-deficient conditions. Analyze the sequences for ORF's and promoter regions. Use DNA's that are specific for ORF's identified in objectives nos. 1 and 2 to probe RNA transcripts made under Mo-deficient conditions. Characterize strains carrying site-specific deletions in the ORF's identified under objectives nos. 1 and 2. Continue purification and characterization of nitrogenase proteins which are expressed by the deletion strain under Mo-deficient conditions.

Approach: DNA sequencing using the dideoxy chain termination method will be used to analyze the genes involved in the alternative N(2) fixation system. Functional genes will be identified by generating mutations with Tanr cartridges. Site specific mutations will also be created using synthetic oligonucleotides. Transcription will be studied using Northern blot techniques. Alternative nitrogenase proteins will be characterized by EPR, EXAFS and neutron activation analysis.

Progress: 88/01 to 88/12. The nucleotide sequence of a region of the Azotobacter vinelandii genome exhibiting sequence similarity to nifH has been determined. The order of open reading frames (ORFs) within this 6.1-kbp region was found to be anfH alternative nitrogen fixation (nifH-like gene), anfD (nifD-like gene), anfG (potentially encoding a protein similar to the product of VnfG and A. chroococcum), anfK (nifK-like gene) followed by two additional ORFs. The 5'-flanking region of anfH contains a nif promoter similar to that found in the A. vinelandii nifHDK gene cluster. The presumed products of anfH, anfD and anfK are similar in predicted Mr and pI to the previously described subunits of nitrogenase-3. Deletion plus insertion mutations introduced into the anfHDGK region of wild-type strain A. vinelandii CA resulted in mutant strains that were unable to grow in Mo-deficient N-free medium, but grew in the presence of 1 uM Na2MoO4 or V2O5. Introduction of the same mutations into the nifHDK deletion strain CA11 resulted in strains that grew under diazotrophic conditions only in the presence of vanadium. The lack of nitrogenase-3 subunits in these mutant strains was demonstrated through two-dimensional gel analysis of protein extracts from cells derepressed for nitrogenase under Mo and V deficiency. These results indicate that anfH, anfD and anfK encode structural proteins for nitrogenase-3.

Publications: 88/01 to 88/12
BISHOP, P. E. et al. 1988. Alternative
nitrogen fixation systems in Azotobacter
vinelandii. 1988. In: Nitrogen Fixation:
Bothe, H., F. (eds.), Gustar Fisher Berlag,
Stuttgart-New York. p. 71-79.

JOERGER, R. D., and P. E. BISHOP. 1988.
Bacterial alternative nitrogen fixation
systems. 1988. CRC Critical Reviews in
Microbiology 16:1-14.

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Nucleotide sequence and genetic analysis of
the nifB-nifQ region from Azotobacter
vinelandii. J. Bacteriol 170:1475-1487.

66.009* CRISO093970
STRUCTURE AND REGULATION OF NIF GENES IN
RHIZOBIUM FREDII, A NEWLY DESCRIBED SPECIES OF
SOYBEAN RHIZ

ELKAN G H; Microbiology; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO3906 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 89

Objectives: To determine the nature of regulation of the nif and nod genes in Rhizobium fredii a new species of soybean rhizobia. To improve biological nitrogen fixation in soybeans by producing and selecting genetic hybrids of R. fredii and Bradyrhizobium japonicum for enhanced symbiotic properties. The long range goal is the development of improved strains of bacteria for use as a legume inoculant.

Approach: The following experiments will be done: isolate total DNA from R. fredii isolates- PRC 206, PRC 206C, PRC 194 and PRC 063, restriction endonuclease digestion of total DNA, Southern hybridization of DNA with labeled pRmR2; isolation of plasmid DNA from PRC 063, restriction endonuclease digestion of plasmid DNA, Southern hybridization with pRmR2 comparing plasmid and total DNA; Northern blot analysis and nitrogenase enzyme level assays; and cloning and sequencing of some regions of chromosomal (integrated) and plasmid borne nif genes.

Progress: 88/01 to 88/12. Rhizobium fredii strain USDA 206 harbors four large plasmids, one of which carries nodulation and nitrogen fixation genes. A previously isolated group of plasmid cured derivatives of strain USDA 206 were compared with each other to determine possible functions for the plasmids. Mutant strain 206CANS was isolated as a non-mucoid derivative of strain 206CA, a mutant cured of two plasmids. The non-mucoid phenotype of 206CANS was only expressed when the strain was grown on certain media, particularly those with polyols as carbon sources. Plasmid pRj206b of strain 206CANS was previously shown to have a higher copy number than the same plasmid in strain USDA 206 and in strain 206CA. This plasmid, when transferred to non-mucoid strains, conferred on recipient strains a non-mucoid phenotype. The symbiotic effectiveness of the wild-type and cured strains was compared. Overall, few differences were shown, but strains 206CA and 206CANS were

found to have higher nitrogenase activity than the other strains. Thus, there appears to be a possible relationship between colony morphology, plasmid copy number, and symbiotic effectiveness.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

66.010* CRISO141623
BIOLOGY, EPIDEMIOLOGY, GENETICS, AND CONTROL OF
VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN

GINGERY R E; LOUIE R; MCMULLEN M; Agricultural Research Service, Wooster, **OHIO** 44691. Proj. No.: 3607-22240-001-00D

Project Type: INHOUSE Agency ID: ARS Period: 01 APR 86 to 31 MAR 91

Objectives: a) Devise maize virus and mycoplasma assays, b) elucidate geographical and biological origins of maize mosaic virus, c) characterize maize viruses, d) establish maize chlorotic dwarf etiology, e) isolate vectors of maize white line and maize subtle mosaic viruses, f) assess yield loss caused by maize viruses, g) elucidate virus disease epidemiology.

Approach: a) Monoclonal antibodies against maize viruses will be used to devise assays, b) world-wide MMV isolates will be compared by a variety of tech- niques and analyzed by cladistic methods to determine phylogeny, c) viruseswill be chemically, physically and serologically characterized to determine their impact on corn production, d tissue will be examined for auxiliary particles by double-stranded RNA analyses and particle isolations, e) sus- pected vectors will be isolated by population dilution and selective growing conditions, f) losses due to virus diseases in widely-used inbreds and hybrids will be assessed, g) disease incidence will be correlated with environment, vector populations, and virus sources.

Progress: 88/01 to 88/12. The chromosomal map position of the major gene for resistance to maize dwarf mosaic virus (MDMV) in the inbred PA405 was determined using restriction fragment length polymorphism analysis. The effect on MDMV-induced symptom expression of transferring this gene into two susceptible backgrounds was also determined. Antibodies specific to each of the three maize chlorotic dwarf virus capsid proteins were used to screen gt10 cDNA expression libraries and putative cDNA clones for each of the capsid proteins were isolated. Maize necrotic lesion virus was purified, and the particles had a similar morphology in EM to those from leaf dip preparations. Maize white line mosaic virus from infected root inocula was consistently transmitted to healthy maize seedlings growing in modified hydroponic conditions. Transmission was unaffected by the fungicides and antibiotics tested. The genes for the capsid and noncapsid proteins of maize strip virus (MStV) were cloned and used to detect the virus in infected plants and insects. A cDNA library to MStV was created and 30 clones of parts of

the MStV genome isolated. A new virus, maize yellow stripe virus, was isolated and characterized.

Publications: 88/01 to 88/12

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Restriction fragment length polymorphism analysis of resistance to maize dwarf mosaic virus. Genome 30, Suppl. 1: 451 (Abstr.).

MILLER, J., OGDEN, S., MCMULLEN, M., HERBERT, A., and STORB, U. 1988. The order and orientation of mouse lambda-rearrangement patterns. J. Immun. 141:2497-2502.

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AMMAR, E.O., and LOUIE, R. 1988. Viruslike

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AMMAR, E.O., GINGERY, R.E., and NAULT, L.R. 1987. Interactions between maize mosaic and maize stripe viruses in their insect vector, Peregrinus maidis, and in maize, Phytopathology 77:1051-1056.

HUNT, R.E., NAULT, L.R., and GINGERY, R.E. 1988. Evidence of infectivity of maize chlorotic dwarf virus and for a helper component in its leafhopper transmission. Phytopathology 78:499-504.

GINGERY, R.E. 1988. The rice stripe virus group. Chapter 9 (pp. 297-329) in: "The Filamentous Plant Viruses" (R.G. Milne, ed.) Vol. 4 in The Plant Viruses (H. Fraenkel-Conrad and R.R. Wagner, eds.) Plenum Press, New York.

66.011* CRISO130207 CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM

PRIBNOW O G; GOLD M H; Oregon Graduate Center, Beaverton, OREGON 97006. Proj. No.: ORER-8602787 Project Type: CRGO

Agency IO: CRGO Period: 30 SEP 86 to 30 NOV 89

Objectives: PROJ 8602787. In order to enhance the potential of Phanerochaete chrysosporium for lignin biodegradation, we need to understand the molecular structure and mechanism of expression of the genes coding for the enzymic components of the degradative system. It is now clear that two P. chrysosporium enzymes, lignin peroxidase (LiP) and manganese peroxidase (MnP), constitute important components of the degradation system. Hence, initially we intend to study the molecular structure and regulation of the genes encoding these enzymes.

Approach: The following experimental approach will be taken. First, using synthetic oligonucleotide probes, assays based on enzyme function, or specific antibody probes we will isolate LiP and MnP genes from genomic and cONA libraries prepared from P. chrysosporium. Then we will determine the ONA sequences of the structural genes and their flanking regions for both enzymes in order to predict amino acid

sequences for the two enzymes and also in order to ascertain important structural features of the genes themselves, including regulatory signals and intron-exon boundaries.

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Progress: 87/01 to 87/12. Purified lignin peroxidase (LiP) and manganese peroxidase (MnP) were used to raise polyclonal antibodies in rabbits. These antibodies were then used to screen a bacteriophage lambda gt11 library for recombinant phages carrying fungal cONA's for these enzymes. A second anti-rabbit-antibody complexed to alkaline phosphatase was used to detect positive clones. More than twenty independent clones were isolated for each of the peroxidases. Lambda ONA was isolated from each clone and the size of each insert was determined to be between 0.6-1.3 Kb. Concurrently, the N-terminal amino acid sequences were determined for purified LiP and MnP proteins. Oligonucleotide probes based on the N-terminal sequences were prepared and used to confirm the identities of clones selected by antibody screening. Several of the large lambda gt11 inserts which hybridize to the oligonucleotide probes will be ligated into a suitable sequencing vector such as M13mp18 or pIBI30 in preparation for sequencing the entire coding regions of the LiP and MnP genes. These cONA clones will also be used to probe a genomic ONA library in order to investigate the extent and size of the postulated gene families coding for LiP and MnP isozymes.

Publications: 87/01 to 87/12
NO PUBLICATIONS REPORTED THIS PERIOO.

66.012* CRISO099027 GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION

KRUL W R; Plant Science; University of Rhode Island, Kingston, **RHODE ISLAND** 02881.

Proj. No.: RIR-8600544 Project Type: CRG0 Agency ID: CRG0 Period: 01 SEP 86 to 31 AUG 89

Objectives: Proj. 8600544. Confirm mode of inheritance and function of resistance genes in Vitis and Nicotiana to wide and limited host range strains of Agrobacterium tumefaciens; determine which pathogen genes are required for infectivity, elicitation of the hypersensitive response; and the modulation of pathogen and host genes by phytohormones.

Approach: Genetics of plant resistance will be determined by conventional breeding methods coupled to screens with wild type and genetically modified pathogen races with or without phytohorome pretreatment.

Progress: 87/10 to 88/09. The interactions of virulence (vir) and tumor inducing (T-SNA) genes of the plant pathogen Agrobacterium tumefaciens and Vitis and Nicotiana species and interspecific hybrids were examined. Incompatible reactions to the wide host range (WHR) stains of the bacterium were conditioned by both dominant and recessive plant genes. The recessive plant gene(s) regulates a hypersensitive reaction in response to wide host range strains. A restriction map of the

limited host range (LHR) vir region has been completed and strains containing lacZ gene from E. coli have been inserted into all vir loci. Some lacZ mutants were marker exchanged into the limited host range bacterium and will be tested for infectivity on grapevine. Inducers of LHR virulence genes from Vitis, Lycopersicon and Nicotiana species are not homologous to the wide host range inducer acetosyringone or to simple phenolic compounds. Compounds that induce WHR strains do not induce LHR strains. All LHR inducers obtained thus far have molecular weights 1000 and induce both LHR and WHR vir loci. Vir gene repressors were obtained from resistant species of Nicotiana and the non host maize. Genetic analysis of a maize mutant (repressor minus and vir inducer(s) plus) shows that a single dominant gene modulates repressor content. Inducer and repressor compounds may modulate infectivity and host selectivity of the pathogen.

Publications: 87/10 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

66.013 CRISO136218
ISOLATING AND CHARACTERIZING A-BETA MATING-TYPE
ALLELES OF SCHIZOPHYLLUM COMMUNE.

ULLRICH R C: Botany; University of Vermont, Burlington, VERMONT 05405.

Proj. No.: VT-B0-00442 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: Examine structure and functioin of A-beta mating-type alleles from the basidiomycete, Schizophyllum commune. Compare the structure of two or more wild-type A-beta alleles and that of an A-betal constitutive mutant allele. Define functional domains of the A-beta locus.

Approach: Isolate A-beta alleles from cosmid libraries. Subclone cosmid inserts isolated to smallest functional units active in transformation. Obtain and analyze DNA sequence. Define functional domains of A-beta locus by in vitro mutagenesis and transformation. Determine if transcript is made from A-beta locus.

Progress: 88/01 to 88/12. This project has been active for only a few weeks.

Publications: 88/01 to 88/12
DRUMMOND, B.J. 1988. Analysis of TRP1 in
 wild-type, trp mutant and trpt transformant
 strains of Schizophyllum commune. M
GIASSON, L., MILGRIM, C., SPECHT, C.A.,
 NOVOTNY, C.P., and ULLRICH, R.C. 1988.
A multiallelic mating-type alleles of the
 basidiomycete, Schizophyllum commune.
 Genome 30:300. Abstr.
PHELPS, L., BURKE, J., ULLRICH, R.C., and

NOVOTNY, C.P. 1989. Nucleotide base sequence of the mitochondrial COIII gene o SPECHT, C.A., MUNOZ-RIVAS, A.M., NOVOTNY, C.P., and ULLRICH, R.C. 1988.
Transformation of Schizophyllum commune: an analysis of parameters for improving transformation frequencies.

SPECHT, C.A., MUNOZ-RIVAS, A.M., NOVOTNY,
C.P., and ULLRICH, R.C. 1989.
Transformation of Schizophyllum commune: an
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ULLRICH, R.C., L. GIASSON, C. MILGRIM, C.A. SPECHT, C.P. NOVOTNY 1988. Isolating mating-type genes of the wood-rotting Basidiomycete and pathogen Schizophyllum commune. Proc. Intl.

ULLRICH, R.C., L. GIASSON, C. MILGRIM, C.A. SPECHT, and C.P. NOVOTNY 1988. Cloning and analysis of A mating-type alleles from Schizophyllum commune. Mating type control in lower e

ULLRICH, R.C., GIASSON, L., MILGRIM, C., SPECHT, C., NOVOTNY, C.P. 1988. Isolation of alpha mating-type genes and directed alteration of mating type of.

66.014 CRISO131841 AMINO ACID METABOLISM AND THE RHIZOBIUM-LEGUME SYMBIOSIS

KAHN M L; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO4772 Project Type: CRG0 Agency ID: CRG0 Period: O1 AUG 86 to 31 MAY 89

Objectives: PROJECT 8601281. The long term objective is to determine the detailed mechanism and regulation of the nutrient exchange in Rhizobium-legume symbiosis. Specific objectives are to investigate the role of amino acid catabolism in symbiotic nitrogen fixation. This approach is based on a nitrogen carrier model for nutrient exchange between the symbionts proposed by the PI.

Approach: Specifically we propose to generate mutants of R. meliloti that have defects in amino acid catabolism, determine the enzymatic defect in these mutants and assess the effect of the mutations on symbiotic nitrogen fixation. Generate mutants of R. meliloti that have defects in intermediary carbon metabolism, determine the enzymatic defect in these mutants and assess the effect of the mutations on symbiotic nitrogen fixation. Continue studies of the genetics of ammonia assimilation. Use the genetic results to predict the pathways of carbon and nitrogen flow in nodules. Assess the possibility that the malate-aspartate shuttle is an important carrier of energy into bacteroids. We will attempt to locate the critical carrier activities in the bacteroid or peribacteroid membrane. Results of these experiments will provide a text of the nitrogen carrier model.

Progress: 88/01 to 88/12. Work has continued in characterizing the role of glutamine synthetases in Rhizobium meliloti. A mutant unable to express GSII has been shown to have a mutation in the R. meliloti ntrA gene. The ntrA mutant blocks the expression of GSII and a GSII-beta-galactosidase hybrid but does not stop the production of GSI. The GSIII activity described below is not found in the ntrA mutant. The DNA sequence of the ntrA gene has been determined. It has been suggested that the glutamine synthetase II gene found in Rhizobium

and Bradyrhizobium was acquired by the bacteria from their host plants. The DNA sequence of the glutamine synthetase II gene from Rhizobium meliloti has been determine and compared to other glutamine synthetases of this type from plants, animals and bacteria. Although the sequence is about 40% identical to plant and mammalian sequences, the two eukaryotic sequences are 50% identical and therefore are more closely related. We are able to explain these results without requiring a novel eukaryotic to prokaryotic transfer of information. We have purified to apparent homogeneity a unique glutamine systhetase (GSIII) from an R. meliloti mutant that lacks GSI and GSII. This protein has high biosynthetic activity but very low transferase activity. Activity is dependent on divalent cations and ATP and is inhibited by methionine sulfoximine, although at concentrations about 100 fold higher than those that inhibit the other GS proteins.

Publications: 88/01 to 88/12

KAHN, M.L., KRAUS, J. and SHATTERS, R.G. 1988. Bacterial Catabolism of Nitrogen-Containing Compounds in Symbiotic Nitrogen Fixation. In Physiological Limitations and The Genetic Improvement of Symbiotic Nitrogen Fixation.

SHATTERS. R.G. 1988. Glutamine Synthesis in Rhizobium meliloti. Ph.O. Thesis, Washington State University.

KERPPOLA, T.K. and KAHN, M.L. 1988. Symbiotic Phenotypes of Auxotrophic Mutants of Rhizobium meliloti 104A14. J. Gen. Micro. 134:193-199.

KERPPOLA, T.K. and KAHN, M.L. 1988. Genetic Analysis of Carbamoylphosphate Synthesis in Rhizobium meliloti 104A14. J. Gen. Micro. 134:921-929.

66.015 CRISO132673
MOLECULAR TAGGING OF THE RP LF GENE IN MAIZE

ELLINGBOE A H; QIN M; ROBERTSON W; Plant Pathology; University of Wisconsin, Madison, **WISCONSIN** 53706.

Proj. No.: WISO3172 Project Type: CRG0 Agency IO: CRG0 Period: 15 JUL 87 to 31 OEC 89

Objectives: PROJ. 8700291. Clone the ONA segments that contain Mu-1. Determine the complementation between descendants of the 38 independently induced mutants.

Approach: A library of mutant 0-4 has been prepared in lambda EMBL3. We are now screening for plaques containing Mu-1. Plaques that contain an Hind III fragment of approximately 7.0 Kb will be used in experiments to determine which contains flanking sequences that segregate with the Rplf gene. Mutants will be intercrossed to determine which mutants will compliment to restore the resistance phenotype.

Progress: 88/01 to 88/12. We have continued to try to determine which of the many copies of Mu is in he Rpl locus. A library of one mutant, 0-4, was prepared in lambda EMBL3. Dut of more than 2.2×10 plaques screened, 78 positives were identified using pMuED2 as probe. ONA

prepared from 42 clones were digested with one or more enzymes, electrophoresed, and probed with pMuEO2. All hybridized with pMuED2 but only 20 hybridized with the internal fragment of Mu1. The blots were also probed with the unique fragments of Mus3, 4, 5, 6, 7, and 8 (obtained from V. Chandler). Four clones hybridized with Mu5 and 3 hybridized with Mu8. Two restriction fragment length polymorphisms (RFLPs) (285 and 422 from NPI) show polymorphisms in the Rp1 region on chromosome 10 to 5 corn lines isogenic except for 5 different alleles at the Rp1 locus. Two probes, prepared by primer extension away from the ends of Mu of two clones 6-1-1 and 6-22, also showed polymorphisms when the DNA of 5 isogenic 1nes was cut with either of 2 enzymes. No polymorphisms among the isogenic lines were observed with probes prepared with the other lambda clones. Spm has now been transferred into plants with Rp10, Rp1e or Rp1k by crossing following a procedure used for Mu1. None has yet been screened for mutations at the Rp1 locus.

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Publications: 88/01 to 88/12

BENNETZEN, J.L., BLEVINS, W.E. and ELLINGBOE, A.H. 1988. Cell-autonomous recognition of the rust pathogen determines Rp1-specified resistance in maize. Science 241:208-210. BENNETZEN, J.L., QIN, M.-M., INGELS, S. and ELLINGBOE, A.H. 1988. Allele specific and mutator-associated instability at the Rp1 disease resistance locus of maize. Nature 332:369-370.

CM 67 PLANTS - NOT COMMODITY ORIENTED

67.001 0090545
EXPRESSION OF MELANIN IN PLANTS - MONITORING
GENE EXPRESSION

BOHNERT H J; Biochemistry; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-136334-H-49-081

Project Type: HATCH

Agency ID: CSRS Period: 01 OCT 89 to 30 SEP 92

Objectives: To construct genes that express a protein that leads to a color change in specific plant organs. Plant transformation and monitoring of gene expression.

Approach: Modified genes will be constructed using different promoter regions that drive expression of a polyphenoloxidase coding region. These genes will be stably transformed into ornamental species. Biochemical characterization of the expressed protein and analysis of the products of enzyme activity will be performed.

Progress: 88/01 to 88/12. We are studying the genome and gene organization of the "ancient" plastid found in Cyanophora paradoxa as a model for shuttling genes and gene products between cellular compartments.

Publications: 88/01 to 88/12

BREITENEDER, H., SEISER, C., LOEFFELHARDT,
W., MICHALOWSKI, C.B., BOHNERT, H.J.
(1988). Physical map and gene map of
cyanelle DNA from the second known isolate
of Cyanophora paradoxa (Kies strain). Curr.
Genetics 13:199-206.

MICHALOWSKI, C.B., RICKERS, J., RAMAGE, R.T., SCHMITT, J.M., BOHNERT, H.J. (1988). Functional replacement in bacteria of a higher plant gene for phosphoenol pyruvate carboxylase. In: Physiol. Suppl. 86:16 (Abstract).

JANSSEN, I., JAKOWITSCH, J., MICHALOWSKI, C., BOHNERT, H.J., LOEFFELHARDT, W. (1988). Sequence Analysis of the Cyanelle PSBA-Gene from Cyanophora paradoxa. The Second International Congress of Plant Molecular Biology, Jerusalem, Nov. 13-18.

67.002 CRISO136882
ROLE OF LOW MOLECULAR WEIGHT HEAT SHOCK
PROTEINS IN PLANT DEVELOPMENT

VIERLING E; Biochemistry; University of Arizona, Tucson, **ARIZONA** 85721. Proj. No.: ARZT-386300-G-49 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 88 to 31 AUG 90

Objectives: PROJ. 8801182. The mRNAs encoding proteins from three gene families of low molecular weight HSPs are present in mature pea (Pisum sativum, cv. "Little Marvel") seeds grown in the absence of heat stress, suggesting HSPs are important for seed development. Each of the HSPs expressed during development will be characterized by DNA sequence analysis of corresponding cDNA clones. To determine when HSPs function, the temporal and spatial regulation of HSP mRNA and protein levels will be examined in developing and germinating pea seeds. Seeds of other plant species, including

Arabidopsis and Petunia will be tested for HSP expression to determine if HSPs are fundamentally important to seed development in many species.

Approach: The HSP cDNA clones have been obtained and will be characterized by standard techniques. Monospecific polyclonal antibodies which recognize each HSP will be generated using fusion proteins produced in E. coli as antigens. Seeds grown in a controlled environment will be analyzed on Northern blots and by in situ hybridization. Western analysis will be used to measure protein levels.

67.003 CRISO132665
GENE ACTIVATION MECHANISMS IN THE INITIATION OF
PLANT DEFENSE RESPONSES

LAMB C J; Plant Biology Lab; Salk Inst For Biological Studies, San Diego, **CALIFORNIA** 92138.

Proj. No.: CALR-8700690 Project Type: CRGD Agency ID: CRGD Period: O1 JUL 87 to 30 JUN 89

Objectives: PROJ. 8700690. The overall aim of this project is to elucidate the molecular mechanisms underlying induction of chalcone synthase (CHS) genes in relation to stimulation of phytoalexin in bean Phaseolus vulgaris L. as a model for the early events related to expression of disease resistance in biologically stressed plants.

Approach: Use of gene transfer techniques in conjunction with precise in vitro mutagenesis to identify specific cis-acting regulatory sequences needed for induction of CHS genes by elicitor and/or infection. Analysis of elicitor-and infection-induced changes in chromatin structure in the region of activated CHS genes to identify specific sequences of regulatory significance. Identify and characterize specific trans-acting regulatory proteins that interact with CHS regulatory sequences.

67.004 CRISO099203
CELLULASE GENE EXPRESSION DURING FRUIT
DEVELOPMENT

CHRISTOFFERSEN R E; Biological Sciences; University of California, Santa Barbara, CALIFORNIA 93106.

Proj. No.: CALR-8600666 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 86 to 31 AUG 88

Objectives: PROJ 8600666. The proposed research will characterize the structure and developmental regulation of cellulase (endo-1, 4-b-glucanase) genomic DNA from both avocado and tomato.

Approach: The avocado cellulase gene will be transferred to the tomato plant with a Ti plasmid derived gene vector, and developing

fruit from the transgenic plants assayed for the expression of the avocado gene. The tomato cellulase gene upstream sequences will be fused to a reporter gene and reintroduced to the tomato plant. Expression of the reporter gene will be followed during fruit development and ripening. Deletion of the regulatory elements and analysis in vivo will eventually define the cis acting elements responsible for the expression these genes during fruit ripening.

67.005 CRISO135659 MUTATIONAL ANALYSIS OF A THYLAKOID MEMBRANE PROTEIN

TDBIN E M; Biology; University of California, Los Angeles, CALIFORNIA 90024.

Proj. No.: CALR-8801177 Project Type: CRGD Agency ID: CRGD Period: 01 AUG 88 to 31 JUL 90

Objectives: PRDJ. 8801177. To isolate a fourth cab gene found in Arabidopsis thaliana and to determine if its expression can, in part, account for the multiplicity of light-harvesting chlorophyll a/b-apoproteins (LHCP) seen in thylakoids of the plant to further define the functional and structural domains of LHCP such as the regions important for thylakoid insertion, chlorophyll binding, and assembly into the holocomplex.

Approach: The DNA corresponding to the fourth hybridizing band on a genomic Southern will be isolated from the corresponding gel and cloned. Clones containing the cab sequence will be selected using a coding region probe. The clone will be characterized by sequencing and, in addition, it will be used to examine the capacity of the corresponding polypeptide to become inserted into thylakoid membranes and to assemble into LHCII by use of an in vitro uptake system in isolated chloroplasts mutant genes synthesized in vitro will be transcribed and translated into the corresponding mutant proteins which will be used in the in vitro uptake system to determine the functional properties of the mutant proteins.

67.006*

CRISO083153

ALLARD R W IN THE GENUS AVENA; Genetics; University of California, Davis, **CALIFORNIA** 95616.

Proj. No.: CA-D*-GEN-4067-H Project Type: HATCH Agency ID: CSRS Period: 01 DEC 80 to 30 SEP 86

Objectives: To determine genome organization and evolution in the genus Avena at the DMA level of resolution: To correlate morphological characters, disease resistance, and other genetic characteristics of wild and domesticated oats with major repetitive DNA families and allozyme profiles.

Approach: The approach will be multi-disciplinary. Data will be collected and integrated from cytology, morphology, protein electrophoresis, and DNA analysis of wild and cultivated oats.

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Progress: 80/12 to 86/09. Research reported earlier in this project established that specific combinations of ribosomal DNA spaces-length variants are correlated precisely with multilocus allozyme genotypes, and with habitat, in California. For example, a "xeric" genotype characterized by specific alleles for eight allozyme loci, and found exclusively in arid habitats is also fixed for rDNA spacer-length variants (alleles) 13, 10, 8 and 7 whereas a "mesic" genotype characterized by a different set of specific alleles for the eight allozyme loci is fixed for rDNA spacer-length variants (alleles 15, 9, 8, and 7). Studies in the present report period have focused on A. barbata from the Mediterranean Basin and especially on Spanish populations known to be ancestral to the colonial California populations. Results establish that all of the allozymes and rDNA alleles from the ancestral Spanish populations are present in California but that none of the multilocus genotypes of California are present in Spain. Thus, during 200 years of evolution in California, selection has arranged the basic genetic materials introduced from Spain into novel sets of coadapted complexes, each precisely correlated with specific habitats in California. Three manuscripts reporting these results are under preparation for publication in Genetics and the Proceedings of the National Academy of Sciences.

Publications: 80/12 to 86/09 ND PUBLICATIONS REPORTED THIS PERIOD.

67.007 CRISO083157 THE EVOLUTION OF GENE LOCI IN PLANTS

GDTTLIEB L D; Genetics; University of California, Davis, **CALIFORNIA** 95616.

Proj. No.: CA-D*-GEN-4066-H Project Type: HATCH Agency ID: CSRS Period: O1 OCT 86 to 30 SEP 91

Objectives: To examine the evolution of duplicate gene loci encoding the cytosolic isozyme of phosphoglucose isomerase (EC 5.3.1.9). We previously completed total purification, structural and immunological comparisons, and analysis of gene dosage. We now propose to determine the nucleotide sequences.

Approach: Standard procedures of molecular biology will be used. The PGA genes will be cloned from a cDNA library, inserted into an expression vector, and identified by antibody screening. The cDNA clones will be used to identify the genes in genomic libraries. The genes and their nontranslated flanking regions will be sequenced and characterized from several species.

Progress: 88/01 to 88/12. Molecular genetic studies of glycolytic phosphoglucose isomerase isozymes (EC 5.3.1.9) in Clarkia (Onagraceae),

a genus of plants native to California, were continued. During the past year we sequenced, for the first time in plants, a nuclear gene encoding the chloroplast isozyme of PGI and one encoding the cytosolic isozyme. The former isozyme governs a reaction preceding the synthesis of starch and the latter one preceding the synthesis of sucrose. The identities of the genes were validated by comparison of their predicted amino acid sequences to recently published sequences for mouse and pig PGIs. The two Clarkia PGI genes are about 60% similar over their entire coding regions and each is about 65% similar to pig PGI. Neither Clarkia gene has introns and both can be expressed in E. coli. The PGI proteins synthesized in E. coli form dimers, are catalytically active and their electrophoretic mobilities are similar to those of appropriate Clarkia PGIs. Since PGI from a prokaryote has not yet been sequenced, and since to study the evolution of this gene a prokaryotic "reference point" was required, we also sequenced the PGI gene from E. coli. The E. coli PGI gene is 88% similar to the sequence of the plant chloroplast PGI and about 60% similar to that of the cytosolic PGI, a result consistent with the model that the chloroplast PGI gene was derived from a prokaryotic source.

Publications: 88/01 to 88/12
TAIT, R.C., FROMAN, B.E.,
LAUDENCIA-CHINGCUANCO, D.L. and GOTTLIEB,
L.D. (1988). Plant phosphoglucose isomerase
genes lack introns and are expressed in
Escherichia coli. Plant Molecular Biology
11:381-388.

67.008 CRISO133265 POLYGALACTURONASE: ENZYME FUNCTION AND GENE REGULATION DURING FRUIT RIPENING

FISCHER R L; Molecular Plant Biology; University of California, Berkeley, CALIFORNIA 94720.

Proj. No.: CA-B*-MPB-4865-CG Project Type: CRG0 Agency ID: CRG0 Period: 15 AUG 87 to 31 AUG 89

Objectives: To understand how the production of polygalacturonase is regulated during tomato fruit ripening. To determine if the action of polygalacturonase, a cell wall degrading enzyme, results in fruit softening. Proj. 8701297.

Approach: The DNA sequences and cellular factors that regulate polygalacturonase gene expression during fruit ripening will be identified. In addition, an inducible polygalacturonase gene will be constructed and inserted into the tomato genome. The effect of induced polygalacturonase gene expression on cell wall structure and softening in unripe fruit will be determined.

Progress: 88/01 to 88/12. Tomato fruit ripening is accompanied by extensive degradation of polyuronide cell wall components by polygalacturonase (PG). To elucidate PG function, we have utilized the rin mutation that blocks many aspects of ripening including the activation of PG gene transcription. The PG

structural gene was ligated to a promoter that is inducible in mature rin fruit. Then rin plants containing the novel E8-PG gene were regenerated. Expression of this gene resulted in the accumulation of active PG enzyme and the degradation of cell wall polyuronides in transgenic rin fruit. However, no effect on fruit softening, ethylene evolution, or color development was detected. These results indicate that PG is the primary determinant of cell wall polyuronide degradation, but suggest that this degradation is not sufficient for the induction of softening or ripening in rin fruit. In addition, we have observed that PG gene expression in rin fruit dramatically increases their susceptibility to pathogen infection, suggesting that this enzyme plays an important role in determining the outcome of host-pathogen interactions.

Publications: 88/01 to 88/12 DELLAPENNA, D., LINCOLN, J.E., FISCHER, R.L. and BENNETT, A.B. (1989). Transcriptional analysis of polygalacturonase and other ripening associated genes in Rutgers, rin, nor and Nr tomato fruit. Plant Physiol. In press. BENNETT, A.B., DELLAPENNA, D., FISCHER, R.L., GIOVANNONI, J. and LINCOLN, J.E. (1989). Regulation, maturation and function of tomato fruit polygalacturonase. In Signals for Cell Separation (Osborne, D., ed.) Springer-Verlag. In press. BENNETT, A.B., DELLAPENNA, D., FISCHER, R.L., GIOVANNONI, J. and LINCOLN, J.E. (1989). Tomato fruit polygalacturonase: gene regulation and enzyme function. In Biotechnology and Food Quality. (Shain-Dow Kung, ed.) University of Maryland Prs.

67.009 0094519 REGULATION OF ETHYLENE INDUCED GENE EXPRESSION DURING FRUIT RIPENING

FISCHER R L; Molecular Plant Biology; University of California, Berkeley, **CALIFORNIA** 94720.

Proj. No.: CA-B*-MPB-4481-H Project Type: HATCH Agency ID: CSRS Period: O1 NOV 84 to 30 SEP 88

Objectives: To identify and analyze genes (and their protein products) whose expression is modulated by the plant hormone ethylene during fruit ripening.

Approach: Clones of mRNAS that accumulate when tomato fruit is exposed to ethylene will be isolated. The number of different induced mRNAs and their abundance will be determined. The patterns of expression of specific ethylene-induced genes will be examined. The protein products of selected genes will be identified and analyzed.

Progress: 84/01 to 88/09. The plant hormone ethylene has a profound influence on fruit ripening. To elucidate the mechanism of ethylene action, we have isolated and analyzed a set of genes that are both developmentally regulated during tomato fruit ripening and ethylene-inducible. We have found that one of these genes encodes a novel proteinase

inhibitor I polypeptide. To begin to identify factors that might regulate their expression, we have identified nuclear proteins that specifically bind to their promoter regions. One factor binds to several ethylene-responsive genes which are coordinately expressed during tomato fruit ripening. Furthermore, both gene expression and DNA-binding activity are activated at the onset of wild-type fruit ripening, and are repressed in ripening-impaired mutant fruit. These results suggest that this DNA binding factor may be involved in the regulation of gene expression during fruit ripening.

Publications: 84/01 to 88/09
LINCOLN, J.E. and FISCHER, R.L. (1988).
Regulation of gene expression by ethylene
in wild type and rin tomato (Lycopersicon
esculentum) fruit. Plant Physiol.
88:370-374.

DEIKMAN, J. and FISCHER, R.L. (1988). Interaction of a DNA binding factor with the 5'-flanking region of an ethylene-responsive fruit ripening gene from tomato. EMBO J. 7:3315-3320.

MARGOSSIAN, L.J., FEDERMAN, A.D., GIOVANNONI, J.J. and FISCHER, R.L. (1988). Ethylene-regulated expression of a tomato fruit ripening gene encoding a proteinase inhibitor with a glutamic residue at the reactive site. Proc. Natl. Sci, USA.

67.010 CRISO134199 THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION

QUAIL P H; Molecular Plant Biology; University of California, Berkeley, **CALIFORNIA** 94720. Proj. No.: CA-B*-MPB-4904-CG Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 87 to 31 AUG 89

Objectives: PROJ. 8702806. To understand the molecular mechanism by which phytochrome regulates plant gene expression.

Approach: The structure of the phytochrome molecule will be studied by sequence analysis. Of particular interest will be a search for conserved features by comparing monocot and dicot sequences. The structure of phytochrome genes will be determined for the purposes of identifying sequences potentially involved in the autoregulation of these genes.

Progress: 88/01 to 88/12. We have initiated experiments to identify regulatory factors that interact with elements in the 5' flanking DNA of the Avena phy-3 gene. The most immediately obvious and attractive candidate for a role in autoregulation of the phy gene is the photoreceptor molecule itself. We have tested for the interaction of purified 124 kDa Avena phytochrome with DNA fragments derived from the phy-3 gene using two different assays. a) Immunoprecipitation of phytochrome from mixtures of the purified photoreceptor and labeled restriction fragments to test for phytochrome-bound DNA; and (b) A gel-retardation assay in which labeled DNA fragments that bind phytochrome are expected to exhibit reduced migration relative to the free

DNA upon electrophoresis through nondenaturing polyacrylamide gels. No detectable binding of the photoreceptor was observed in either the Pr or Pfr forms, to any phy gene sequence tested, from 1 kbp upstream to approximately 130 bp downstream of the transcription start site. We are also using gel retardation assays to test for factors extracted from Avena seedling nuclei capable of binding to phy-3 promoter DNA. Preliminary data indicate the presence of a factor(s) that binds to restriction fragments upstream of the transcription start site. The degree of interaction appears to be unaffected by red-light irradiation 2 h before extraction.

Publications: 88/01 to 88/12
QUAIL, P.H., GATZ, C., HERSHEY, H.P., JONES, A.M., LISSEMORE, J.L., PARKS, B.M., SHARROCK, R.E., BARKER, R.F., IDLER, K., MURRAY, M.G., KOORNEEF, M., KENDRICK, R.E. (1987). Molecular biology of phytochrome. In Phytochrome and photoregulat.

67.011* CRISO134506 ISOLATION OF A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO

WILLIAMSON V M; Nematology; University of California, Davis, **CALIFORNIA** 95616. Proj. No.: CA-D*-NEM-4920-H Project Type: HATCH Agency ID: CSRS Period: 12 APR 88 to 30 SEP 92

Objectives: To clone the Mi gene which confers resistance to rootknot nematodes in tomato. To investigate the mechanisms of resistance. To transfer Mi to other plant species.

Approach: We will clone Mi by virtue of its linkage to the gene Aps-1 which encodes acid phosphatase-1. Acid phosphatase-1 will be purified and used to produce antibody and obtain peptide sequence. We will screen a cDNA library for a clone of Aps-1 and use this clone to "walk" to Mi. We will transform susceptible tomato with Mi candidates and assay for resistance to nematodes. An approach to Mi using transposon tagging is also planned. The level, localization and regulation of Mi expression will be determined. Mi will be transferred to other plant species using Agrobacterium based vectors.

Progress: 88/04 to 88/12. Progress has been made toward our goal to clone the tomato gene Aps-1 which encodes acid phosphatase-1. We have purified the enzyme acid phosphatase-1 from cell suspension culture of the nematode resistant tomato cultivar VFNT cherry. We have cleaved the purified protein with proteases and have obtained amino acid sequence information on some of the peptides obtained. This sequence information was used to generate three oligonucleotide probes and these were used to screen a VFNT genomic library. Seven candidate clones have been obtained and are being analyzed to see if they correspond to Aps-1. The Aps-1 clone will be used as a starting point in our attempt to clone the nematode resistant gene Mi of tomato by chromosome walking.

Publications: 88/04 to 88/12

No publications reported this period.

67.012* CRISO136124 MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO

WILLIAMSON V M; Nematology; University of California, Davis, **CALIFORNIA** 95616.

Proj. No.: CA-D*-NEM-5001-CG Project Type: CRGO Agency ID: CRGO Period: O1 JUL 88 to 30 JUN 91

Objectives: PROJ. 8800667. Mi is a dominant locus that confers resistance to root-knot nematodes when present in tomato. Our goal is to clone Mi to increase understanding of the mechanism of resistance conferred by this gene.

Approach: Mi is closely linked to Aps-1, encoding acid phosphatase-1 in tomato. Acid phosphatase-1 will be purified from cell suspension culture and used to produce antibody and obtain peptide sequence for identification of the corresponding cDNA. Using this clone and clones of other linked fragments (RFLPs) as probes, DNA from resistant and susceptible cultivars will be analyzed. Candidate clones of Mi will be obtained by "chromosome walking" techniques. We propose to identify Mi by complementation of function after transformation of susceptible tomato cultivars with candidate clones using Agrobacterium based vectors.

Progress: 88/08 to 88/12. We have obtained data that will be of value in our attempt to clone the nematode resistance gene Mi of tomato by chromosome walking from the linked gene Aps-1. Using DNA clones which flank the region of Chromosome VI, which carries Aps-1 and Mi, 7 and 11 genetic map units away, we examined DNA from various tomato cultivars. Southern blot analyses were carried out using DNA from tomato cultivars that differ in the Aps-1 alleles, and in whether they carried Mi. Our results indicate that the size of the region of the tomato genome derived from the wild tomato species L. peruvainum (the source of Mi) varies among cultivars. This region of DNA is quite extensive in some cultivars, where it includes a DNA marker that is 7 map units away from Aps-1. This information will help us to localize the region of DNA encoding Mi for our chromosome walking experiments.

Publications: 88/08 to 88/12
No publications reported this period.

67.013 CRISO135934 ORGANIZATION AND STABILITY OF GENES FOR RESISTANCE AND AVIRULENCE

MICHELMORE R W; Vegetable Crops; University of California, Davis, **CALIFORNIA** 95616.

Proj. No.: CA-D*-VCR-4994-CG Project Type: CRGO Agency ID: CRGO Period: O1 JUL 88 to 30 JUN 91

Objectives: PROJ. 8800574. To conduct a detailed genetic analysis of genes for resistance to characterize their organization

and stability.

Approach: Three hypotheses will be tested using restriction fragment length polymorphisms and pulsed field gel electrophoresis. 1) Resistance genes are located in hotspots of meiotic recombination and unequal crossing-over. 2) Resistance genes are unstable and exhibit a high mutation rate. 3) Genes determing field resistance are similar to major genes for resistance but are of minor effect.

Progress: 88/01 to 88/12. The project involves the completion of genetic linkage maps for lettuce and its fungal pathogen, Bremia lactucae and use of the markers to study genetic mechanisms generating variation at resistance (Dm) and avirulence (Avr) loci (mainly the former). The study integrates classical genetics, molecular markers (restriction fragment length polymorphisms, RFLPs), and pulsed field gel electrophoresis (PFGE). The overall genetic maps are nearing adequate density. We are now focusing on identifying markers tightly linked to Dm genes by screening bulks of random cDNA clones against near-isogenic lines. Several markers have been identified this way. The first map was generated from an intraspecific cross. We are now analyzing a wide interspecific cross. This should allow us to coalesce the linkage groups and also study the map compression associated with the interspecific nature of the cross. Very high molecular weight plant DNA has been isolated, restricted, separated by PFGE, and hybridized to single-copy RFLP probes. Several chromosomes of B. lactucae have been separated by PFGE, isolated from the gel and cloned to make chromosome-specific libraries as sources of probes for chromosome-walking to clone Avr genes. To test for the spontaneous mutation rate of Dm genes, 50,000 F(subscript 1) lettuce seed was generated which is heterozygous for several Dm genes. These will be screened by simultaneously inoculating with several isolates each detecting a single gene during the next year.

Publications: 88/01 to 88/12
No publications reported this period.

67.014 CRISO033901 CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS

STERN H; Contract & Grant Admin; University of California-san Diego, La Jolla, CALIFORNIA 92093.

Proj. No.: 8200489 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 82 to 28 FEB 85

Objectives: Proj. No. 8200489. The research is addressed to a molecular analysis of meiosis in Zea mays. The immediate goals of the program involve: Identification and characterization of Zea genomic clones housing meiosis-specific sequences; comparative analyses of relevent genomic regions in normal and mutant meiotic types; examination of transcriptional lesions in meiotic mutants using cloned meiosis-specific cDNA probes.

Approach: The experimental approach is based upon the use of meiosis-specific cloned cDNA probes that have been or will be developed in the research program on meiosis in Lilium.

Progress: 87/01 to 87/12. Dur project involves the identification and examination of recombinant DNA clones from Zea mays for sequences active during meiosis, employing cDNA clones prepared from the biochemically tractable Lilium system. Work has focused in particular on a large repeat sequence family, the EMPR (expressed meiotic prophase repeats), the sequences of which are coordinately transcribed into poly A(+) RNA during the meiotic interval in microsporocytes. We have successfully identified EMPR-homologous maize genomic clones and subcloned their homologous regions in plasmid vectors. The homology is strongest over a segment corresponding to the least diverged portion seen among subgroups of the family in lily. Probes for this region cross-react to meiotic-stage-specific $\bar{1}$ ily RNA with the same profile as lily EMPR cDNA clones & to RNA prepared from maize spikelets containing meiotically active microsporocytes. Direct sequence analyses and comparisons now indicate that these sequences in maize and lily encode messages for acidic meiotic proteins. We are currently examining the tissue and cellular specificity of RNA for these clones, and also non-meiosis-specific clones, in maize, in order to compare their expression pattern with that seen in lily. The presence of multiple copies of these segguences in these evolutionarily diverged species, and the similarities in their pattern of expression, suggest an important, possibly structural, role for their products in meiosis.

Publications: 87/01 to 87/12

ND PUBLICATIONS REPORTED THIS PERIOD.

67.015 CRISO099980 MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND DEVELOPMENT

BRAY E A; Botany & Plant Sciences; University of California, Riverside, **CALIFORNIA** 92521. Proj. No.: CA-R*-BPS-4754-H Project Type: HATCH Agency ID: CSRS Period: O1 DCT 86 to 30 SEP 91

Objectives: Study the mechanism that results in expression of specific gene products in response to plant growth substances; determine the role of the specific gene products in plant growth and development. Provide information needed for programs designed to select or genetically modify crop plants for improved yield in favorable and nonfavorable environments.

Approach: Identify the DNA sequences that confer ABA responsiveness to ABA-regulated storage protein genes in developing soybeanseeds. Determine if ABA-binding proteins are involved in this response. Identify and characterize genes and gene products that are expressed in response to ABA during environmental stress within vegetative and reproductive plants.

Progress: 88/01 to 88/12. Many plants respond to drought stress by accumulating the plant hormone abscisic acid (ABA) and several specific proteins and mRNAs. An ABA-deficient mutant of tomato, in which ABA accumulation does not occur during drought stress, was used to demonstrate that ABA synthesis during stress is required for the accumulation of several of these drought-induced proteins and mRNAs. However, heat shock proteins were synthesized in the ABA-deficient mutant and in the wild type, suggesting that ABA does not play a major role in induction of gene expression during heat stress. A cDNA library, constructed from mRNA isolated from drought stressed tomato leaves, was screened by differential hybridization. cDNAs that corresponded to mRNAs that were expressed in drought stressed wild type plants and not in drought stressed ABA-deficient plants were identified. Three unique cDNAs were isolated that correspond to mRNAs that are only induced during drought stress in plants that synthesize ABA. These cDNAs are also induced by ABA in the mutant and the wild type. Therefore, the mRNAs that correspond to the isolated cDNAs are induced in response to the ABA that is synthesized during drought stress.

Publications: 88/01 to 88/12
BRAY, E.A. (1988). Drought- and ABA-induced changes in polypeptide and mRNA accumulation in tomato leaves. Plant Physiol. 88: 1210-1214.
BRAY, E.A. (1988). Altered polypeptide biosynthesis in drought-stressed ABA-deficient tomato mutants. J. Cell. Biochem. 12C: 208 (abstract).
BRAY, E.A. (1988). Regulation of gene expression by abscisic acid in drought-stressed tomatoes. 13th Int. Conf. Plant Growth Substances, Calgary, Canada, Abstr. 128.

67.016 CRISO136664 REGULATION OF GENE EXPRESSION BY ABSCISIC ACID DURING DROUGHT STRESS

BRAY E A; Botany & Plant Sciences; University of California, Riverside, **CALIFORNIA** 92521. Proj. No.: CA-R*-BPS-5040-CG Project Type: CRGD Agency ID: CRGO Period: O1 SEP 88 to 31 AUG 90

Objectives: PRDJ. 8801205. The molecular mechanism of gene induction by abscisic acid (ABA) in drought-stressed plants will be determined. ABA-induced genes will be isolated and ABA-responsive DNA sequences within these ABA-induced genes will be identified.

Approach: A cDNA library will be constructed from mRNA isolated from drought-stressed tomato leaves and will be screened with wild type and an ABA-deficient mutant. cDNAs that are only expressed in the wild type are potentially induced by ABA. ABA-induced genes will be isolated from a genomic library. The ABA-induced promoters will be spliced onto a reporter gene and these chimeric genes will be transferred into tomato using Agrobacterium. The expression of the gene under the control of the ABA-induced promoter will be characterized.

67.017 CRISO096565 MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES

CLEGG M T; Botany & Plant Sciences; University of California, Riverside, **CALIFORNIA** 92521. Proj. No.: CA-R*-BPS-4563-H Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: Determine the extent and distribution of DNA sequence diversity in germplasm collections of several major crop plants; determine the categories of mutational events which contribute to genetic diversity in domesticated plants; use molecular markers to map the genome of major crop plants; determine the biosystematic relationships between cultivated plants and their wild relatives.

Approach: DNA sequence diversity will be investigated for nuclear and organelle genomes in crop plants. Methods include restriction fragment analysis of organelle genomes, analysis of restriction site polymorphism via Southern hybridization experiments, cloning specific plant genes, and determining the complete DNA sequence of specific genes.

Progress: 88/01 to 88/12. Studies of molecular genetic diversity in crop plants have the following four objectives: (1) to conduct an extensive characterization of levels of diversity in avocado (Persea americana) germplasm; (2) to perform a detailed analysis of molecular variants in pearl millet (Pennisetum glaucum) and maize (Zea mays); (3) to characterize chloroplast DNA (cpDNA) polymorphisms in barley (Hordeum vulgare); and (4) to undertake a genetic and molecular characterization of flower color polymorphisms in the morning glory (Ipomoea purpurea). With respect to objective 1, approximately 30 DNA samples have been purified from avocado cultivars and from various hybrids between cultivars. Considerable RFLP variation has been detected and in many cases this variation allows the discrimination of cultivars within varieties. Work on objective 2 has concentrated on a characterization of molecular diversity at the DNA sequence level. The gene coding for Adh 1 has been cloned from pearl millet and will be sequenced. In addition, approximately four Ds1 elements have been cloned from the genome of maize and will be subjected to further analysis. The focus of objective 3 is a precise analysis of cpDNA polymorphisms in barley using PCR amplified DNA sequences. Work on objective 4 has concentrated on the genetic characterization of a mutator element that causes high frequencies of somatic and germline mutation in loci that determine flower color in the morning glory.

Publications: 88/01 to 88/12

EPPERSON, B.K. and CLEGG, M.T. (1987).

Instability at a flower color locus in the morning glory. J. Heredity 78: 346-352.

GERLACH, W.L., DENNIS, E.S., PEACOCK, W.J. and CLEGG, M.T. (1987). The Ds1 controlling element family in maize and Tripsacum. J. Mol. Evol. 26: 329-334.

CLEGG, M.T. and EPPERSON, B.K. (1988).

Natural selection of flower color polymorphisms in morning glory populations.

Chapter 10. In: Gottlieb, L. and Jain, S.K., eds. Plant Evolutionary Biology. Chapman-Hall Ltd., London. p. 255-273. EPPERSON, B.K. and CLEGG, M.T. (1988). Genetics of flower color polymorphism in the common morning glory (Ipomoea purpurea). J. Heredity 79: 64-68. LEARN, G.H., DURBIN, M.L. and CLEGG, M.T. (1988). A gene for tRNA-Ile(CAU) from the chloroplasts of a monocot, Pennisetum americanum. Nucleic Acids Res. 16: 4734. GEPTS, P. and CLEGG, M.T. (1989). Genetic diversity in pearl millet (Pennisetum glaucum (L.) R. Br.) at the DNA sequence level. J. Heredity. In press.

67.018 CRISO090517 DEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELD AND GENE EXPRESSION

ROOSE M L; Botany & Plant Sciences; University of California, Riverside, CALIFORNIA 92521.
Proj. No.: CA-R*-BPS-4362 Project Type: STATE Agency ID: SAES Period: 01 JUN 83 to 30 SEP 88

Objectives: Develop methods by which genes controlling quntitatively inherited traits can belocalized to chromosome segments and manipulated in breeding programs; use lines containing these genes to increase understanding of responses to selection for improved yield and quality traits and to locate genes controlling expression of structural genes.

Approach: Identify inbred lines which differ at 20 or more isozyme or DNA sequence marker loci and for quantitative characters, tranfer chromosome segments marked by each locus to one line by repeated backcrossing and selection. Self pollinate lines and select progeny homozygous for each marker. Test lines for yield and other agronomic traits and for differences in protein expression.

Progress: 83/06 to 86/09. Major accomplishments of the project were in defining the extent of genetic diversity in isozymes and seed proteins among cultivars of cowpea (Vigna unguiculata (L.) Walp), tomatillo (Physalis philadelphica Lam.), and sesame (Sesamum indicum L.). In cowpea, there was virtually no genetic variation among 13 morphologically and geographically diverse cultivars at 35 isozyme loci. There was more variation in molecular weight of seed proteins, with clear variation at 2 loci and occasional variants for a few additional bands among 139 cultivars. Other Vigna species differed from V. unguiculata at many loci. Populations of tomatillo were found to have considerable genetic diversity at isozyme loci, but the mating system was shown to be virtually 100% outcrossing. Therefore, the objectives of this study could not be pursued with this species because it would be very difficult to develop near-isogenic lines. Sesame is predominantly self-pollinated, and easily crossed. Among 30 cultivars, breeding lines, and accessions tested for variation in 16 enzyme systems, there was variation at 2 alcohol dehydrogenase loci. Variation at IDH and PGI was also observed, but did not appear

to be stably expressed. Variation at seed proteins was studied using SDS-PAGE and isoelectric focusing, but little variation was observed. The level of variation in sesame and cowpea was too low for the objectives of the project to be achieved with these species. Future research on sesame will be conducted under 3898-RR.

Publications: 83/06 to 86/09 ND PUBLICATIONS REPORTED THIS PERIOD.

67.019 CRISO135594
MOLECULAR BIOLOGY OF HOST-PARASITE INTERACTIONS
BETWEEN ROOT-KNOT NEMATODES AND PLANTS

BIRD D M; Nematology; University of California, Riverside, **CALIFORNIA** 92521. Proj. No.: CA-R*-NEM-4968-H Project Type: HATCH Agency ID: CSRS Period: 22 AUG 88 to 30 SEP 92

Objectives: The goal of this project is to understand the mechanism by which root-knot nematodes induce and maintain cyncytia in the root tissue of their host. Specifically, I plan to characterize nematode exudates, the signals that trigger exudate production and the transcriptional events that occur in root tissue during nematode infection.

Approach: Antibodies raised against nematode exudates will be used to select the genes encoding these proteins from a genomic expression library. Genomic clones will be characterized by standard methods, including DNA sequencing. Expression of these genes will be examined, and selected genes will be expressed in bacteria for injection experiments. Differential cDNA libraries also will be constructed from normal and infected root tissue.

Progress: 88/08 to 88/12. Mutations in the Caenorhabditis elegans dpy-13 (dumpy) gene result in a short, chunky body shape. This gene was tagged by insertion of the Tc1 transposon, and the wild-type gene was cloned by chromosomal walking 11 kb from ama-1, a cloned gene encoding the large subunit of RNA polymerase II. Three transposon insertion sites in dpy-13 are located near the 5' end of a 1.2 kb transcribed region. The EMS-induced reference allele, dpy-13(e184), carries a small deletion near the middle of this gene. The DNA sequence reveals that dpy-13 is a member of the collagen multi-gene family, and it could encode a polypeptide of 302 amino acids. A 146 base pair sequence, encoding amino acids 56-103, is unique in the C. elegans genome, and it hybridizes to a 1 kb mRNA of moderate abundance.

Publications: 88/08 to 88/12
VON MENDE, N. BIRD, D.M. ALBERT, P.S. AND RIDDLE, D.L. (1988). dyp-13: A nematode collagen gene that affects body shape. Cell, Vol. 55:567-576.

67.020 CRISO097155
FUNCTIONAL AND MUTATIONAL ANALYSIS OF A
THYLAKOID PROTEIN

TDBIN E M; KDHDRN B D; University of Los Angeles, Los Angeles, **CALIFORNIA** 90024. Proj. No.: CA-8502776 Project Type: CRGD Agency ID: CRGO Period: O1 SEP 85 to 29 FEB 88

Objectives: Proj 8502776. The synthesis and assembly of chlorophyll-protein complexes of higher plants are intimately related to the development of chloroplasts. We plan to study the production and integration of members of such a complex, the light-harvesting chlorophyll a/b-proteins (LHCP), into thylakoid membranes.

Approach: We plan to study the structural and functional domains of the LHCP in a biologically significant environment by examining mutant LHCP in thylakoid membranes. We have made mature peptide coding sequence deletions by in vitro mutagenesis, and we wish to study the interaction of the mutant proteins with the chloroplast. These studies will involve both chloroplast uptake of purified precursor LHCP in vitro, and the expression and utilization of LHCP DNA sequences upon introduction into tobacco plants with a Tiplasmid based vector.

Progress: 85/09 to 87/12. Two different Lemna gibba genes encoding light-harvesting chlorophyll a/b-proteins (LHCP) differ substantially in their transit peptides: however, both share a framework of amino acids common to a number of nuclear encoded chloroplast proteins. We demonstrated that both kinds of transit sequences can function in an in vitro uptake system. We sequenced three linked genes encoding the LHCP in Arabidopsis thaliana and found that they encode identical mature apoproteins. Because a fourth hybridizing band appears on genomic Southern blots, we believe this species has a fourth related gene, but we have not yet been successful in isolating a clone containing it. We suspect that it may account in part for the heterogeneity observed in the LHCPs found in Arabidopsis thylakoid membranes. We used mutant proteins created in vitro to try to understand the structural and functional domains of the LHCP apoprotein. We made deletions and point mutations of the Lemna gibba AB30 gene and several fusions of portions of this protein with a ribulose 1,5-bisphosphate carboxylase (rubisco) small subunit sequence from Lemna. We transcribed (with SP6 polymerase) and translated (with wheat germ) the altered sequences to give us the corresponding mutant proteins. These mutant proteins were characterized in an in vitro chloroplast uptake system. We found that several of the deletion mutants could still be inserted into the thylakoids, but not assembled into LHCII.

Publications: 85/09 to 87/12
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Transit peptides of nuclear-encoded
chloroplast proteins share a framework of
homologous amino acids. EMBD J. 5, 9-13.

- KOHDRN, B.D., HAREL, E., CHITNIS, P.R., THDRNBER, J.P. and TOBIN, E.M. 1986. Functional and mutational analysis of the light-harvesting chlorophyll a/b protein of thylakoid membranes. J. Cell Biol. 102, 972-981.
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67.021* CRISO140448 WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS

GREENE F C; ANDERSDN D D; CAMPBELL D E; Plant Physiology & Chemistry Research Unit; Western Regional Res Center, Albany, **CALIFORNIA** 94710. Proj. No.: 5325-41000-007-00D

Project Type: INHDUSE Agency ID: ARS Period: O1 DCT 85 to 30 SEP 89

Objectives: To identify regions of the wheat genome that control the timing and specific localization of storage protein gene expression in the wheat seed; to determine the molecular basis of such control, and its potential application for improvement of wheat protein quality.

Approach: An interdisciplinary approach involving molecular biology, plant physiology and genetics will be utilized in this research. The structural organization of wheat chromosomal DNA in storage protein genetic loci, including both genes and intergenetic regions, will be determined via DNA hybridization and sequence analysis. Model systems, utilizing transfer of DNA to heterologous and homologous cells, will be developed for the study of wheat storage protein gene expression, and to identify potential regulatory genes. The effects of in vitro DNA sequence modification on theexpression of these genes will be studied at levels of gene transcription/ mRNA synthesis and protein synthesis.

Progress: 88/01 to 88/12. Completed sequencing of the six high-molecular-weight glutenin genes from the wheat cultivar Cheyenne. Continued analysis of the alpha-gliadin wheat storage protein genes and the low-molecular-weight glutenin genes. The LMW studies are continuing in cooperation with University of California, Davis. Completed construction of a large wheat lambda library to

be used in specific sequence isolation. Initiated a study of the wheat sucrose synthase genes by isolating two genomic clones homologous to a maize sucrose synthase DNA fragment. Completed an analysis of the optimization of heterologous expression in yeast of a wheat alpha-gliadin gene. Concluded that optimimum expression of the construct was obtained by control of the carbon source and by modifying yeast plasmid constructs carrying the wheat gene to increase alpha-gliadin gene copy numbers. Began the introduction of site-specific changes in storage protein genes for high-level expression in both yeast and E. coli: succeeded in changing sequence sites in a HMW gene to isolate the coding sequence. Initiated tissue culture and plant cell protoplast preparation, and began studying the control of wheat storage protein gene chimeric constructs. Preliminary data suggest positive activating factors control these genes. Initiated cooperative work with Cornell on transformation and regeration of rice with wheat storage protein gene contructs.

Publications: 88/01 to 88/12

ANDERSDN, D.D., HALFDRD, N.G., FDRDE, J.,
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high-molecular-weight glutenin genes from
Triticum aestivum L. cv. Cheyenne. Proc.7th
Int.Wheat Gen.Sym.Acc.7/13/88.

GREENE, F.C., ANDERSDN, D.D., YIP, R.E., HALFDRD, N.G., MALPICA-RDMERD, J.-M., and SHEWRY, P.R. '988. Analysis of possible quality related sequence variations in the 1D glutenin HMW subunit in genes of wheat. Proc.7th.Int.Symp.Acc.7/13/8.

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ANDERSDN, D.D., YIP, R.E., HALFDRD, N.G., FDRDE, J., SHEWRY, P.R., MALPICA- RDMERD, J.-M. and GREENE, F. 1988. Nucleotide sequences of 2 HMW glutenin genes from the D-genome of a hexaploid bread wheat. Nucl.Acids Res. Acc. 11/28/88.

ANDERSDN, O.D. and GREENE, F.C. 1988. The characterization and comparative analysis of HMW, glutenin genes from genomes A & B of a hexaploid bread Conclu ded that optimimum expression of the construct was obtained by control of the.

67.022* CRISO137480 BARLEY GENETICS AND PLANT CYTOGENETICS

TSUCHIYA T; HANG A; WANG S; Agronomy; Colorado State University, Fort Collins, **COLORADO** 80523.

Proj. No.: CDL00625 Project Type: HATCH Agency ID: CSRS Period: 14 FEB 89 to 30 JUN 93

Objectives: The overall objective of this project is basic genetic and cytogenetic studies in various plant species for aiding the progress in genetics and their direct and/or indirect uses in plant breeding programs.

Approach: We use chromosome manipulation approaches in most of the research work. For barley genetics chromosomal mutants, mainly various types of trisomics and many genetic mutants are used to improve genetic linkage maps and study the genetic architecture of barley chromosomes. For other materials, karyotype analysis by conventional and/or Giemsa-banding techniques of chromosome studies will be used.

67.023 CRISO136163 REGULATION OF GRASSHOPPER REPRODUCTION BY JUVENILE HORMONE

ROBERTS P E; Entomology; Colorado State
University, Fort Collins, COLORADO 80523.
Proj. No.: COLO0685 Project Type: HATCH
Agency ID: CSRS Period: O1 OCT 88 to 30 JUN 92

Objectives: To isolate mRNA from the fat body of Melanoplus bivittatus. To separate mRNAs by size class on gels. To select mRNAs that are present only when juvenile hormone stimulates the fat body. To produce cDNA from the mRNAs transcribed in response to juvenile hormone. To isolate and purify genomic DNA. To isolate genes complementary to the cDNA probes.

Approach: Gene cloning techniques will be used to isolate regulatory sequences recognized by juvenile hormone receptors. These sequences are used in the control of reproduction and development of insects. The regulatory sequences will be compared with those of insects that have other forms of JH, and means to modify these sequences to control reproduction will be sought.

67.024 CRISO033736 DEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON

MENZEL M Y; Biological Sciences; Florida State University, Tallahassee, **FLORIDA** 32306.

Proj. No.: 8100239 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 81 to 31 DEC 84

Objectives: Develop a system for generating and using deficiencies for all of the individual chromosomes of the genome or Upland cotton (Gossypium hirsutum L.).

Approach: The viable duplication - deficiencies that can be recovered from heterozygotes of 58 lines homozygous for reciprocal exchanges between non-horologous chromosomes are being systematically generated and characterized as to their extent, frequency and morphological and cytological stigmata. The use of deficiencies thus generated for mapping ganes and linkage groups and for rapid introduction of foreign genes is also being initiated.

Progress: 85/01 to 85/12. Mapping of 116 breakpoints (bp's) in 58 translocation lines involving simple reciprocal exchanges has been completed and a chromosome map based on chiasma frequencies has been constructed. Mapping the bp's nearly triples the number of chromosome reference points available in cotton. All arms of the A(h) genome are marked by at least one bp, but 8 or 9 arms of the D(h) genome have no bp's in this set of translocations. The ratio of A(h) to D(h) bp's (1.7:1) corresponds exactly to the estimated amount of DNA in A(h) and D(h) (Edwards et al. 1974, Chromosoma 47:309-326; Kadir 1976, Chromosoma 56:85-94). The correspondence suggests that the bp's are randomly distributed according to the unit length of DNA. But on the chiasma mpa, the bp's are strongly clustered in the proximal half of the chromosome arms (96% of A(h) and 87% of D(h) bp's). Randomly distributed breaks can be reconciled with proximal clustering on the map if (1) some breaks occurred in proximal heterochromatin, where chiasmata are rare (contracting the map relative to physical length of the chromosome segment near the centromere), and (2) there is strong distal localization of one chiasma per arm (expanding the map relative to physical lenght of the segment at the chromosome ends). The total length of the cotton genomes was estimated to be 3016 cM (A(h) = 1510 cM, D(h) = 1506 cM; ca.60 chiasmata per pollen mother cell). The greater amount of DNA in A(h) is not reflected in the recombination map length.

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Publications: 85/01 to 85/12

MENZEL, M.Y., NAQI, S. and BROWN, M.S. 1984.

Incipient genome differentiation in

Gossypium. IV. The genome of G. laxum. J.

Hered. 75: 389-391.

MENZEL, M.Y., RICHMOND, K.L. and DOUGHERTY,

B.J. 1985. A chromosome translocation

break-point map of the Gossypium hirsutum

genome. J. Hered. In Press (Nov.-Dec.,

1985).

67.025 CRISO134322 TRANSCRIPTIONAL REGULATION OF PLANT HEAT SHOCK GENES

GURLEY W B; Microbiology & Cell Science; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-MCS-02714 Project Type: STATE Agency ID: SAES Period: 22 FEB 88 to 30 SEP 93

Objectives: The primary goals are to characterize cis- and trans-acting factors that regulate thermoinducible transcription of two soybean heat shock genes, Gmhsp17.5-E and Gmhsp26-A.

Approach: To identify the promoter elements by linker scan and deletion mutagenesis using T-DNA-based vectors; to assess the contribution of individual bases using site-directed mutagenesis; to characterize the properties of cis-acting elements with regard to polarity and position; to characterize nuclear proteins that specifically interact with functional domains of heat shock/stress promoters.

Progress: 87/10 to 88/09. Five major sites of transcriptional control have been identified within the 5' flanking sequences of soybean stock (hs) gene Gmhsp17.5-E. These sites correspond to the TATA motif (-30), a high homology heat shock consensus clement (HSE) at site 1 (-60), a low homology HSE at site 2 (-95), an AT-rich sequence at -135, and a TATA/dyad at position -220. Four of these sites have demonstrated transcriptional activity based on results of 5' and internal deletion studies. The AT-rich site has been identified only by in vitro binding of a soybean protein from nuclear extracts. We have characterized the interaction of nuclear proteins with specific DNA sequences upstream of the start site of transcription by DNase I footprinting and gel mobility retardation assays. The AT-rich binding factor (ATBF) is very abundant and is also present in maize coleoptiles and roots, and in pea plumules. Oligonucleotide affinity chromatography is being employed for the purification of the ATBF.

Publications: 87/10 to 88/09
CZARNECKA, E., NAGAO, R.T., KEY, J.L. and
GURLEY, W.B. Characterization of Gmhsp26-A,
A Stress Gene Encoding a Divergent Heat
Shock Protein of Soybean:
Heavy-Metal-Induced Inhibition of Intron
Processing, (1988).

CZARNECKA, E., KEY, J.L. and GURLEY, W.B. Regulatory Domains of the Gmhsp17.5-E Heat Shock Promoter of Soybean: A Mutational Analysis, (Submitted).

67.026* CRISO089967 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT

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GURLEY W B; INGRAM L O; INGRAM L O; Microbiology & Cell Science; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-MCS-O2317 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 82 to 30 SEP 88

Objectives: Identification and characterization of agriculturally important genetic systems. Regulation of gene expression and the delivery of genetic material to higher plants and associative microorganisms.

Approach: Agriculturally important genes in the areas of hydrogen uptake in nodulated legumes, alcohol tolerance in microorganisms, and UV radiation damage will be identified and characterized using a variety of genetic and biochemicals methods. The regulation of the transcriptional aspects of gene expression in higher plants will be studied using the T-DNA of Agrobacterium tumefaciens Ti plasmid as model plant genes. The Ti plasmid will also be utilized in the development of a vector for the introduction of genetic material into plant cells.

Progress: 87/10 to 88/09. We have cloned a positive element that resides upstream of the Adh-1 promoter of maize. This TATA-distal promoter element (located from position -410 to -140) contributes 50% of the transcriptional activity of this gene. We are currently

analyzing the effects of distance, position, and polarity of orientation on transcriptional activity. We have also constructed a series of vectors that will test the effect of substitution of the 780 gene activator (T-right pTi 15955) on core the promoter of Adh-1. The promoter constructs will be expressed transgenetically in sunflower tumors. S1 nuclease hybrid protection mapping of poly(A) RNA will be used for analysis of mutant transcriptional activity.

Publications: 87/10 to 88/09 NO PUBLICATIONS REPORTED THIS PERIOD.

67.027 CRISO099527 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT

GUY C L; Ornamental Horticulture; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-ORH-02317 Project Type: HATCH Agency ID: CSRS Period: 01 DEC 85 to 30 SEP 88

Objectives: Identification and characterization of agriculturally important genetic systems. Regulation of gene expression and the delivery of genetic material to higher plants and associative microorganisms. Somatic cell genetics and plant development: the modification, selection, regulation, and propagation of plants through cell and tissue culture.

Approach: cDNA libraries will be constructed from poly (A) RNA isolated from freezing-tolerant, cold-acclimated tissues.in lambda gt 10. The libraries will be screened with p. cDNAs and low temperature specific clones identified. These clones will be characterized; restriction fragment mapping, base sequence, the size of the mRNA it hybridizes with, and the factors that control the synthesis of its corresponding mRNA. Putative cold hardiness genes will be transferred into freezing sensitive plants to determine effectiveness in increasing freezing tolerance.

Progress: 87/10 to 88/09. During the past year we were able to establish that Citrus sinensis leaves exposed to 5 C contain a 160 KDa protein not present in leaves of plants grown at 25 C. The electrophoretic characteristics of the citrus leaf protein are nearly identical with a protein found in spinach leaves and hypocotyls. The spinach protein is present only in cold acclimated leaves and in increased levels in cold acclimated hypocotyls. We believe the citrus and spinach proteins will be one in same. In the citrus relative, Poncirus trifoliata, cold acclimation of very young seedlings results in increased levels of an mRNA for a 160 KDa protein. While the pl of this protein is different from the spinach and citrus proteins, the similarities of the Poncirus protein suggests it may be an electrophoretic variant of the other proteins. Interestingly, the mRNA for the 160 KDa protein in Poncirus is present in both leaf and stem tissue, both of which were tolerant to freezing stress to -18 C. In

other work, a cDNA library was prepared with poly(A+)RNA from Poncirus leaf tissue. Randomly selected clones are being used to develop a genetic map of cold tolerance genes in a hybrid population of a cross between Poncirus and Citrus grandis.

Publications: 87/10 to 88/09

GUY, C.L., HASKELL, D. and YELENOSKY, G. 1988. Changes in freezing tolerance and polypeptide content of Spinach and Citrus at 5_oC. Cryobiology 25, 264-271.

67.028 CRISO089965 CELLULAR AND MOLECULAR GENETICS FOR CROP **IMPROVEMENT**

FERL R J; VASIL I K; VASIL V; Vegetable Crops; University of Florida, Gainesville, FLORIDA 32611.

Proj. No.: FLA-VEC-02317 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 82 to 30 SEP 88

Objectives: Identification and characterization of agriculturally important genetic systems. Regulation of gene expression and the delivery of genetic material to higher plants and association microorganisms. Somatic cell genetics and plant development: the modification, selection, regulation, and propagation of plants through cell and tissue culture.

Approach: Recombinant DNA cloning and sequence analysis will be used to elucidate the relationship of DNA sequence and gene regulation in several crop species. Cloned genes will be used for in vitro transfer between species in order to develop methodologies for the transfer of agronomically important genes. Cell and protoplast culture techniques will be used to develop the ability to genetically modify and produce superior cereal and grass plants.

Progress: 87/10 to 88/09. Promoter strengths of two maize alcohol dehydrogenase genes Adhl and adh2, and the maize shrunken-1 gene, Sh1, were evaluated by transient expression in cultured protoplasts of Panicum maximum, Triticum monococcum, and Daucus carota. Promoter elements were ligated in correct and opposite orientations as transcriptional gene fusions to the chloramphenical acetyl transferase gene containing the nopaline synthese 3' polyadenylation signal. The relative levels of gene expression were compared to the cauliflower mosaic virus 35S promoter. The full length Adhl promoter (-1100 to +15) functioned in all species, but at a reduced level in D. carota. An Adhl promoter delection from -304 to -1100 did not express at detectable levels in any species nor did the Sh1 promoter construction. The Adh2 promoter (-860 to +90) only expressed in D. carota. The full length Adh1 promoter gave the highest level of CAT expression in the monocot cells but at levels which were approximately 30% compared to the CaMV 35S promoter. This was reduced further in D. carota to approximately 4%. These data suggest that at least some of the regulatory factors responsible for promoter

function are somewhat species specific and that these differences should be considered in gene expression studies.

Publications: 87/10 to 88/09 HAUPTMANN, R.M., AHSRAF, M., VASIL, V., HANNAH, L.C., VASIL, I.K. and FERL, R.U. 1988. Promoter strength comparisons of maize shrunken 1 and alcohol dehydrogenase 1 and 2 promoters in mono- and decotyledonous species. Plant Physiol.

67.029 CRISO142410 CHARACTERIZATION OF MUSA CLONES AND SPECIES USING ISOZYMES AND ORGANELLE DNA MARKERS

JARRET R L; Agricultural Research Service,

Experiment, **GEORGIA** 30212. Proj. No.: 6607-21000-001-04T

Project Type: INHOUSE Agency ID: ARS Period: 01 JAN 88 to 01 JAN 90

Objectives: To clarify the taxonomic relationship of Musa species, to examine their evolutionary relationship with cultivated bananas and plaintains and to utilize genetic/biochemical markers for clonal identification. The marker systems to be employed will include a range of isozyme markers in conjunction with organelle RFLPs. The data will be compared with that collected via standard morphological analysis.

Approach: Plant material (leaf) from Musa species, now in germplasm collections around the world, will be collected and frozen (-135 degree). Samples will be analyzed for enzyme polymorphisms and organelle DNA RFLPs using techniques developed by R. Jarret. Data collected will be numerically analyzed and compared with recent morphological data on same plant material. Data will be used to define systematic relationships and for clonal identification.

Progress: 88/01 to 88/12. A post-doctoral fellow was hired in December 1988 to initiate work on this project. To date, a cDNA library has been constructed for use with this genus using a malaccensis subspecies. DNA sequences have been isolated and cloned in E. coli. Present activities are focused on the use of biotin-labelled probes for the detection of single and low-copy number sequences in total DNA extracts from various clones.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

67.030 CRISO131245 MITOCHONDRIAL DNA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRID CELLS OF GRASSES

OZIAS-AKINS P J; Horticulture; Georgia Coastal Plain Expt Sta, Tifton, GEORGIA 31793. Proj. No.: GE000423 Project Type: HATCH Agency ID: CSRS Period: O1 APR 87 to 31 MAR 90 **Objectives:** Determine if a somatic hybrid cell line consists of a population of cells with varying mitochondrial genomes which develop over time as a result of tissue culture-induced rearrangement and if stress affects frequency or type of mtDNA rearrangements.

Approach: Protoclones will be obtained from suspension-cultured somatic hybrid cell lines and mtDNA restriction patterns will be compared with total cell populations.

Suspension-cultured cells will be subjected to chemical and anaerobic stress to determine if any effects can be observed in the mtDNA restriction patterns with particular emphasis on specific, cloned sequences known to be amplifiable. New somatic hybrid cell lines will be examined to establish temporal changes in mtDNA after cell fusion.

Progress: 88/01 to 88/12. Twenty-five protoclones have been isolated from a two-year old somatic hybrid cell line of Pennisetum americanum + Panicum maximum (SH3). The mtDNA restriction patterns of the protoclones differ from one another with certain enzyme-probe combinations. A change in the number of BamHI fragments hybridizing with a cloned 5.1 kb-BamHI fragment from SH3 was apparent in all protoclones, and this rearrangement must have occurred before the protoclones were isolated. Twenty-four protoclones contained BamHI fragments of 8.0kb, 7.4kb, 6.0kb, 5.1kb, and 0.9kb that hybridized with the cloned 5.1kb-BamHI fragment; one protoclone contained an additional band of 2.8kb. The 8.0kb, 7.4kb, and 0.9kb fragments have been cloned and mapped. The 7.4, 5.1, and 0.9kb fragments hybridize with a 350bp BamHI-SstI fragment containing internal coding sequence from maize atpA. Restriction fragment length polymorphisms could be detected in 12 out of 22 protoclones using two restriction enzymes and a 4.1 kb-PstI fragment cloned from one SH3 cell line. The 4.1kb fragment exists infrequently as a plasmid DNA and more frequently in an "integrated" form. Several of the "novel" bands observed in ethidium bromide-stained gels have homology to the 4.1kb-PstI fragment. Rearrangements involving sequences homologous to this fragment occur frequently during extended culture of the protoclones.

Publications: 88/01 to 88/12 OZIAS-AKINS, P., TABAEIZADEJ, Z., PRING, D.R., VASIL, I.K. 1988. Preferential amplification of mitochondrial DNA fragments in somatic hybrids of the Gramineae. Curr. Genet. 13:241-245. OZIAS-AKINS, P. and VASIL, I.K. 1988. In vitro regeneration and genetic manipulation of grasses. Physiol. Plant 73:565-569. HAUPTMANN, R.M., VASIL, V., OZIAS-AKINS, P., TABAEIZADEH, Z., ROGERS, S.G., FRALEY, R.T., HORSCH, R.B. and VASIL, I.K. 1988. Evaluation of Selectable markers for obtaining stable transformants in the Gramineae. Plant Physiol. 86:602-606. OZIAS-AKINS, P., DUJARDIN, M., HANNA, W.W. and VASIL, I.K. Quantitative variation recovered from tissue cultures of an apomictic, interspecific Pennisetum hybrid. Maydica (in press).

67.031* CRISO130942
IMPROVING THE EFFICACY OF BACULOVIRUS
PESTICIDES BY RECOMBINANT DNA TECHNOLOGY

MILLER L K; Entomology; University of Georgia, Athens, **GEORGIA** 30602.

Proj. No.: GEO-RC293-110 Project Type: CRG0 Agency ID: CRG0 Period: O1 OCT 86 to 30 SEP 87

Objectives: PROJECT 8603163. Improve the efficacy of insect baculoviruses as biological pesticides by introducing insect behavior-modifying genes into baculoviruses. Use recombinant DNA technology to genetically construct a baculovirus that expresses a foreign gene which affects insect behavior.

Approach: Construct recombinant baculoviruses that carry a gene encoding an insect-specific neurotoxin and produce large quantities of toxin in infected insect cells. Determine if the recombinant virus is a more effective biological pesticide and if it has an extended host-range. Study possible strategies for reducing recombinant virus persistance in the environment to enhance the ecological safety of the pesticides.

Progress: 86/10 to 87/09. A gene encoding an insect-specific neurotoxin of scorpion venom, the Buthus eupeus insectotoxin I gene (BeIt1), has been synthesized from oligoneucleotides based on the published amino acid sequence of the toxin. The synthetic gene was cloned in E. coli and the sequence of the gene was confirmed by DNA sequencing. The BeIt1 gene was then transferred to an E. coli gene expression vector utilizing the Tac promoter to drive expression. No toxin expression was observed above background endotoxin activity in E. coli. The BeIt1 gene was transferred to a baculovirus expression system utilizing the polyhedrin promoter to drive toxin expression. No toxin activity was observed in recombinant BeIT1 baculovirus-infected cells. Toxin gene expression in infected insect cells was monitored at the protein synthesis level by pulse-labeling proteins with radioactive methionine. Only very low levels of a new 3.7 kilodalton protein was observed in the recombinant virus-infected cells. It is likely that the BeIT1 toxin is highly unstable under the conditions of expression used and if this gene is to be used to enhance baculovirus pesticide efficacy, a means of stabilizing the small polypeptide will need to be developed. The safety of recombinant baculoviruses with respect to mammalian species was also investigated during the course of this work.

Publications: 86/10 to 87/09

CARBONELL, L.F. and MILLER, L.K. 1987. Appl. Environ. Microbioly, 53:1412-1417. Baculovirus interaction with nontarget organisms: A virus-borne reporter gene is not expressed in two mammalian cell lines. CARBONELL, L.F. and MILLER, L.K. 1987. Genetic engineering of viral pesticides: Expression of foreign genes in nonpermissive cells. In "Molecular Strategies for Crop Protection".

MILLER, L.K. 1987. Expression of foreign genes in insect cells. In "Biotechnology Advances in Invertebrate Pathology and Cell

Culture" (ed. K. Maramorosch). Academic Press, Orlando, Fl.

67.032 CRISO135751 A PLANT ACTIN GENE SUPERFAMILY: AN ASSAY FOR GENES ENCODING NOVEL PHENOTYPES

MEAGHER R B; Genetics; University of Georgia, Athens, **GEORGIA** 30602.

Proj. No.: GEOR-8800501 Project Type: CRG0 Agency IO: CRG0 Period: 15 JUN 88 to 31 MAY 91

Objectives: PROJ. 8800501. The primary objective of this proposal is to determine the mechanisms which led to the amplification of actin genes at several independent locations in the petunia genome and determine the physical size of these amplified blocks of actin genes. The second objective is to develop a novel technique using Class II restriction fragment length polymorphisms (RFLPs) to measure the distance between a DNA probe and a linked genetically characterized phenotypic marker.

Approach: These objectives will be met using the characterized actin gene subfamilies within the actin superfamily in petunia and a combination of classical genetics, RFLP analysis and pulse field gel electrophoresis.

67.033 CRISO132686 MUTAGENIC ANALYSIS OF ANAEROBIC INDUCTION IN PLANTS

STRPMMER J N; Genetics; University of Georgia, Athens, ${\it GEORGIA}$ 30602.

Proj. No.: GEOR-8701000 Project Type: CRG0 Agency IO: CRG0 Period: O1 AUG 87 to 31 JUL 89

Objectives: Proj. 8701000. To sequence Petunia Adh genes and compare them to other plant Adh genes. To characterize the expression of Petunia Adh genes. To assess the ability of portions of the maize Adhl allele to function approximately in petunia.

Approach: Sequencing will be accomplished using nested deletions in M13 and the Sanger dideoxy-method. Analysis will be accomplished using an Intelligenetics Bion Workstation. Chimeric petunia-maize genes will be constructed using recombinant ONA techniques. Expression of petunia and chimeric genes will depend on run-on transcription assays, Northern hybridization, labelling of nascent peptides in vivo, and in vitro translation of RNA. Pulse labelling of plant tissue will also be attempted.

Progress: 88/01 to 88/12. In the first year of funding we have constructed a genomic library from Petunia hybrida (V30); sequenced one of the Adh genes, including 1 kb of flanking sequence; subcloned a second Adh gene and prepared for its sequencing; analyzed the expression of Petunia Adh by means of in vivo labeling of nascent polypeptides, Western Blot analysis, and RNA hybridizations. ELISA assays

are underway, as are efforts to determine which gene encodes which polypeptide by means of RFLP analysis, which analysis has so far permitted the mapping of one gene to Ch 4. The anaerobic response in Petunia is in some ways similar to that of maize: normal translation ceases almost immediately, and a few polypeptides are then synthesized in the root. However, in Petunia the response appears even stronger in shoots and leaves than in roots, where a reasonable level of constitutive expression exists under all the growth conditions we have used. The experiments of the coming year, especially those exchanging promoters of maize and petunia Adh, will be especially important in determining whether the leaky expression in root and strong response in leaf is due to Adh sequences or to a significantly different signalling system. There is also preliminary evidence for a post-translational inhibition of AOH activity in Petunia roots, predicted from differences in measurements in homogenates compared to activity assayed in electrophoretic gels. We have developed the tissue culture and transformation systems.

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Publications: 88/01 to 88/12

HUANG, S., GREGERSON, R. and STROMMER, J. The Anaerobic Response of Maize and Petunia, ms. in preparation.

GREGERSON, R. and STROMMER, J. Molecular Characterization of Petunia Adhl, ms. in preparation.

GREGERSON, R. and STROMMER, J. Structure, Organization and Expression of the Adh Genes of Petunia. ms. in preparation.

67.034 CRISO095600 MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES

OENNY T P; Plant Pathology; University of Georgia, Athens, **GEORGIA** 30602.

Proj. No.: GE000863 Project Type: HATCH Agency IO: CSRS Period: O1 JUL 85 to 31 OEC 88

Objectives: Investigate the genetic difference between P. syringae pv. tomato and P. syringae pv. syringae. Investigate the molecular basis of pathogenicity of P. solanacearum.

Approach: Examine the proteins that are being made by representatives of several P. syringae pathovars using SOS-polyacrylamide gel electrophoresis. Plasmid content and sizes will be determined on agarose gels. Genomic ONA will be isolated from these strains and compared for homology using two different methods. Attempts will be made to detect ONA fragments shared by all selected PST strains that can be purified and subcloned into plasmid vectors. An attempt will also be made to identify the PST genes for host range. A library of P. solanacearum ONA will be made. E. coli recombinants that have the genes for cellulase and polygalacturonase will be identified, and then genes for EPS synthesis will be identified. After mapping and subcloning, each virulence gene will be mutagenized with the transposon Tn5.

Progress: 85/07 to 88/12. Pseudomonas syringae results: The phenotypic and genetic diersity within the pathovar P. s. tomato was found to be low, except for the large variation in plasmid content. This contrasted with the small scale study that suggested large variation in P. s. syringae. P. s. tomato was also shown to be phenotypically and genetically distinct from P. s. syringae, and should probably be considered as a separate species. Techniques of using restriction fragment length polymorphisms (RFLPs) to quantify genetic relationships was developed for this work and applied to a study of 20 P. s. pathovars, and an in depth study of P. s. syringae was initiated. A DNA hybridization probe was developed that differentiated between P. s. tomato and P. s. syringae; this probe might be useful as a rapid diagnostic method. A gene library of P. s. syringae was produced that was used by other researchers to find a unique set of genes involved in pathogen/host interactions. Pseudomonas solanacearum results: A number of virulence genes were cloned from P. solanacearum. The egi gene encodes an endoglucanase enzyme, and Pg1A encodes a major polygalacturonase enzyme; specific inactivation of either gene reduces the rate that wilt symptoms appear but not number of plants killed. A 50 kilobase region that has genes involved in production of extracellular polysaccharide (EPS) was also cloned. Tn5 mutations at two locations, designated epsA and epsB, reduce production of EPS in vitro.

Publications: 85/07 to 88/12

- DENNY, T.P. 1988. Phenotypic diversity in Pseudomonas syringae pv. syringae. J. Gen. Microbiol. 134:1939-1948.
- DENNY, T.P., GILMOUR, M.N., and SELANDER, R.K. 1988. Genetic diversity and relationships of two pathovars of Pseudomonas syringae. J. Gen. Microbiol. 134:1949-1960.
- DENNY, T.P. 1988. Differentiation of Pseudomonas syringae pv. tomato from P. syringae pv. syringae with a DNA hybridization probe. Phytopathology 78:1186-1193.
- DENNY, T.P., MAKINI, F.W. and BRUMBLEY, S.M. 1988. Characterization of Pseudomonas solanacearum Tn5 mutants deficient in extracellular polysaccharide. Mol. Plant Microbe Interact. 1:215-223.
- ROBERTS, D.P., DENNY, T.P. and Schell, M.A. 1988. Cloning the egl gene of Pseudomonas solanacearum and analysis of its role in phytopathogenicity. J. Bacteriol. 170:1445-1451.
- SCHELL, M.A., ROBERTS, D.P. and DENNY, T.P. 1988. Analysis of the Pseudomonas solanacearum polygalacturonase encoded by pg1A and its involvement in phytopathogenicity. J. Bacteriol. 170-4501-4508.
- HUANG, H.-C., SCHUURINK, R., DENNY, T.P., ATKINSON, M.M., BAKER, C.J., YUCEL, I., HUTCHESON, S.W. and COLLMER, A. 1988.

67.035 CRISO098209 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT

Objectives: Identification and characterization of agriculturally important genetic systems.

Approach: Investigate the genetic differences between Pseudomonas syringae pv. tomato (PST) and Pseudomonas syringae pv. syringae (PSS). Investigate the molecular basis of pathogenicity of Pseudomonas solanacearum.

Progress: 87/01 to 87/09. Pseudomonas syringae results. Further work on the project to examine diversity within P. syringae pv. syringae resulted in a new classification scheme for the plasmids of this bacterium that is based both on molecular weight and on DNA homology among the plasmids. A new method of analyzing restriction fragment length polymorphisms (RFLPs) was developed that generates dendrograms based on the calculation of genetic distances and cluster analysis. The dendogram based on RFLP analysis closely matched that produced from protein polymorphism analysis. These types of genetic analysis of will have a major impact on the systematics of pathogenic bacteria. In unrelated research, a gene library was made from a P. syringae pv. syringae strain for use by collaborators at the University of Maryland, who subsequently recovered an unprecedented clone bearing genes involved in pathogenicity and hypersensitive reaction (an hrp gene cluster) that is expressed in E. coli. Pseudomonas solanacearum results. The P. solanacearum mutants deficient in endoglucanase (EGase) and polygalacturonase (PGase) activity were studied further, and their reduced virulence confirmed. Altered growth potential in vitro was not a factor. It was found that EGase activity was confined to the cytoplasm in E. coli, but that most of the PGase activity was secreted into the periplasm. An EGase-, PGase-double mutant was constructed and is currently being characterized.

Publications: 87/01 to 87/09

- HUANG, H.-C., SCHUURINK, R.C., DENNY, T.P., BAKER, C.J., ATKINSON, M.M., HUTCHESON, S.W. and COLLMER, A. 1987. ar cloning of Pseudomonas syringae pv. syringae genes that c
- ROBERTS, D.P., DENNY, T.P. and SCHELL, M.A. 1987. Cloning the egl gene of Pseudomonas solanacearum and analysis of its role in phytopathogenicity. J. Bacteriol. Submitted.
- SCHELL, M.A., ROBERTS, D.P. and DENNY, T.P. 1987. Analysis of the spontaneous mutation to avirulence by Pseudomonas solanacearum. p. 61-66. In D.P.S. Verma and N. Brisson (ed.), Molecular Genetics of Plant-Microbe Interactions.

67.036 GENETICS OF ASPERGILLUS FLAVUS CRIS0095599

PAPA K E; Plant Pathology; University of Georgia, Athens, **GEORGIA** 30602.

Proj. No.: GED00864 Project Type: HATCH Agency ID: CSRS Period: 01 JUL 85 to 30 SEP 86

Objectives: To sample natural populations of A. flavus. To conduct heterokaryon compatibility tests and identify h-c groups. To synthesize heterokaryons and heterozygous diploids for parasexual genetic analyses. To identify and map genes controlling heterokaryon incompatibility.

Approach: Variability in natural populations of A. flavus will be determined from the amount of heterokaryon incompatibility found among isolates. Cultures will be obtained from infected ears of corn randomly selected from narrow and broad geographical areas. In order to test for heterokaryosis, mutants unable to utilize various nitrogen sources will be selected. Part-wise combinations of complementary auxotrophs will be tested for heterokaryon compatibility. Protoplast fusion techniques will be followed to form heterokaryons between incompatible isolates. Parasexual "crosses" to multiple-marked tester strains will be used to identify and map h-c

Progress: 85/07 to 86/09. Strains of Aspergillus flavus were recovered from corn samples from 15 counties in Georgia and placed into 22 different heterokaryon-compatibility (h-c) groups. There was no clear pattern of association between h-c group and aflatoxin or sclerotium production. Strains within the same h-c group were not restricted to the same geographical area. Partial mapping of linkage group II has been accomplished. The sequence of five gene loci on the same chromosome arm has been determined. These mapped genes include one involved in aflatoxin biosynthesis, one for conidial color, and three auxotrophs. Map distances could not be determined.

Publications: 85/07 to 86/09
 PAPA, K.E. 1986. Heterokaryon incompatibility
 in Aspergillus flavus. Mycologia 78:98-101.

67.037 CRISO134697 GENETIC DIVERSITY AND DEGREE OF RELATEDNESS OF PATHOGENIC AND NONPATHOGENIC POPULATIONS OF FUSARIUM

PDPE D D; Plant Pathology; University of Georgia, Athens, **GEORGIA** 30602.

Proj. No.: GE000501 Project Type: HATCH Agency ID: CSRS Period: 01 JUN 88 to 31 MAY 93

Objectives: The objectives of this investigation of Fusarium oxysporum are to provide a profile of the genetic architecture of pathogenic and nonpathogenic populations to be used by breeders as baseline data from which to predict shifts in the genetic structure of the pathogen, and, to resolve taxonomic relationships among populations of Fusarium oxysporum at the DNA level.

Approach: Classical and molecular genetic techniques will be employed, including parasexual, mtDNA RFLP, whole chromosome RFLP, and karyotype analyses of pathogenic and nonpathogenic populations of Fusarium oxysporum. A measure of quantitative and qualitative aspects of genetic diversity among and within populations will be analyzed statistically.

Progress: 88/01 to 88/12. The three phases of this research project have been underway for approximately one year. A reliable technique for exttracting total DNA from Fusarium oxysporum f.sp. lycopersic (FDL) isolates was developed. A genomic library was constructed with DNA extracted from FDL isolate HMS-3. From this library, 18 unique clones have been screened with which to probe restricted genomic DNA of FD1 variants. A nonradioactive DNA labeling technique, involving digoxigenin-dUPT and detection of hybrids by enzyme immunoassay, is being investigated, and will be utilized in Southern blot analyses. RFLPs detected by this method will be analyzed statistically to determine the genetic diversity and the degree of relatedness of the isolates. Pulsed field electrophoresis techniques are being developed to elucidate the FDL karyotype. FDL mycelia in the log phase of growth were embedded in an agar matrix. The cell wall was removed enzymatically with Novozyme-234. Membranes were dissolved with SDS and the liberated genomic DNA electrophoresed on a TAFE pulse field electrophoresis unit. Chromosome bands were detected after staining with ethidium bromide. Electrophoresis parameters are being investigated to obtain optimal band resolution and clarity. UV induced auxotrophic and drug resistance FDL mutants are being generated to investigate the parasexual cycle. Various mutants differing with respect to VCG, race and deographic location will be paired and scored for anastomosis.

Publications: 88/01 to 88/12
 ND PUBLICATIONS REPORTED THIS PERIOD.

67.038 CRISO093261 CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES CODING FOR SPECIFIC GENES

STILES J I; Botany; University of Hawaii, Honolulu, **HAWAII** 96822.

Proj. No.: HAW00655-G

Project Type: SPECIAL GRANT Agency ID: CSRS Period: O1 JUL 84 to 30 JUN 87

Objectives: The overall objectives are to isolate and characterize specific maize genes with the aim of acquiring information necessary for future applied genetic engineering. The specific aims are: to isolate certain specific maize genes from a genomic clone bank and to characterize these genes by DNA sequence analysis, analysis of transcriptional regulation and by genetics.

Approach: A recombinant library of maize will be screened for specific genes using molecular hybridization probes. These probes will be the corresponding gene from another organism, cDNA

clones of specific maize genes and genes differentially expressed during development. These genes will be analyzed by DNA sequencing and by transcript mapping. Gene copy number will be assessed by probing Southern blots of genomic DNA digested with various restriction endonucleases. Transcriptional regulation will be investigated using northern blots of RNA from various tissues grown under various conditions.

Progress: 84/07 to 87/06. A recombinant DNA expression vector was constructed and used to express, in yeast, cDNA clones synthesized using total mRNA isolated from maize seedlings. It was anticipated that by selecting for biological complementation of specific mutations in the host yeast strains, clones containing copies of the corresponding maize gene could be identified. Although a total of 20,000 cDNA clones were analyzed for complementation to 5 yeast loci that encode enzymes in the four different pathways, no complementation was observed. However, the vector is functional and a yeast strain was developed that expresses alpha-gliadin, a wheat seed storage protein. The successful expression of this wild-type gliadin gene is a first step in the development of heterologous expression systems capable of producing gliadins, and other seed storage products, that have been genetically engineered for improved nutritional content or unique baking properties. About 30 mutants that give the defective kernel (dek) phenotype have been isolated from maize lines with active Mul transposable elements. These mutants show decreased transcription of several developmentally regulated genes. Recombinant DNA libraries have been constructed for two of these mutants that are non-allelic and clones containing the transposable element Mul have been isolated. Mapping is currently underway to identify clones containing the dek genes.

Publications: 84/07 to 87/06

1)

NEILL, J.D. 1985. Construction of a novel vector for the expression of foreign genes in yeast. Ph.D Dissertation. Indiana State Univ., Terre Haute. 193p.

WILLIAMS, C.E., et al. 1987. Molecular analysis of defective kernel mutants derived from lines exhibiting Robertson's mutator activity. Journal of Cellular Biochemistry. 11B:(Supplement) 25.

67.039* CRISO134015 REGULATION OF EXPRESSION OF THE BACULOVIRUS, ACNPV

FRIESEN P D; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843. Proj. No.: IDAO0908 Project Type: HATCH Agency ID: CSRS Period: O1 JAN 88 to 06 JAN 89

Objectives: The long term objective of this proposal is to genetically engineer the baculoviruses for improved efficacy as biological control agents of insect pests. Our immediate goals are to: determine the organization of several early viral genes for the insertion of foreign genes, locate those DNA sequences responsible for early and

regulated expression of such genes, and examine the function of the early genes determing whether they are nonessential and are therefore replaceable. This proposal also examines the nature and mutagenic effects of an insect-derived transposable element which has integrated into the DNA genome of the baculovirus, AcNPV. Transposable elements act to decrease the virus' ability to produce its occluded form thereby reducing viral pesticide effectiveness.

Approach: Viral gene organization and the location of DNA control regions will be analyzed by fusing specific genes to easily assayed reported genes and testing them for proper expression in transient assays and by placing them back into the viral genome. Antibodies raised to trihybrid viral fusion proteins will be used to examine viral gene function. The gene organization and mutagenic effects of the transposable element will be determined by DNA sequence analysis and nucleic acid hybridization techniques.

Progress: 88/01 to 88/12. Progress in our investigation of the molecular mechanisms involved in the regulation of baculovirus gene expression has been the identification of DNA sequences responsible for conferring early and late transcription of the gene encoding a 35,000 - molecular - weight protein (35K) in the HindIII-K genome region of Autographa californica nuclear polyhedrosis virus (AcNPV). Mutagenesis of the 35K gene promoter previously linked to the reporter gene for chloramphenicol acetyl-transferase, indicated that sequences from -155 to -55 relative to the RNA start site (position +I) controlled early transcription while sequences from -55 to -4 controlled late transcription in recombinant viruses. Thus, two distinct regions of the 35K gene promoter are involved in early versus late regulation. Both regions contain sequences found at the promoter of other AcNPV genes with similar regulation and may therefore represent common control sequences. These studies on the nature of ACNPV promoters provide necessary information for the construction of recombinant baculoviruses expressing insecticidal genes for improved biological control of insect pests.

Publications: 88/01 to 88/12
NISSEN, M.S., and FRIESEN, P.D., (1989).
Molecular Analysis of the Transcriptional
Regulatory Region of Early Baculovirus
Gene. J. Virology (in press).

67.040* CRISO083485 ORGANIZATION AND EXPRESSION OF A BACULOVIRUS DNA GENOME

MILLER L K; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843. Proj. No.: IDAO0801 Project Type: HATCH Agency ID: CSRS Period: O1 JUL 84 to 30 JUN 89

Objectives: The long-term objectives of this proposal are to improve the efficacy of viral pesticides by genetic engineering technology and facilitate the commercial production of these viruses. To achieve these objectives,

more information conce ning viral gene organization and the regulation of gene expression is required. The immediate goals are, therefore, to locate key viral genes with respect to a physical map of the DNA genome, test an early promoter for controlling early expression of a passenger gene, and define the nature of genes involved in controlling gene expression. Since a handicap in the commercial production of viruses in cell culture was traced to the insertion of mobile genetic elements into the viral DNA.

Approach: Key genes of the baculovirus AcNPV will be mapped with respect to the established physical map of the viral DNA by marker rescue and by cloning cDNAs of early and intermediate viral mRNAs. Detailed knowledge of the nature of one such gene will be obtained; its promoter will be fused to an easily assayable gene and tested for temporal regulation. The position preferences of transposable element insertions will be determined and their effect on viral gene expression will be determined by nucleic acid hybridization techniques.

Progress: 86/01 to 86/12. Research has progressed in several different areas relevant to baculovirus gene organization and expression that are applicable to development of more effective viral pesticides. First, we have synthesized and cloned complementary DNA from 20 different regions of the viral genome. Temporal expression of RNA from each region was examined and all were found to contain overlapping sets of RNA. Many of these overlapping sets of RNA have common 5' or common 3' termini, a common motif in the organization and expression of baculovirus genes. These studies are important since an understanding of the regulation of viral expression is required before the virus can be successfully engineered (via recombinant DNA) as improved pesticides. Secondly, to better understand this regulation, we have analyzed the structure of viral DNA during the viral replication cycle. We found that the viral genome adapts a nucleosomal-like structure typical of DNA undergoing active transcription. Thirdly, we have continued studies on the molecular biology of a transposable element (TED) which inserted into the baculovirus genome causing mutations. This led to the discovery that TED is a member of a newly characterized class of mutagenic elements which resemble the RNA tumor viruses.

Publications: 86/01 to 86/12

WILSON, J. and MILLER, L.K. 1986. Changes in the nucleoprotein complexes of a baculovirus DNA during infection. Virology 151:315-328.

MAINPRIZE, T.M., LEE, K.-J. and MILLER, L.K. 1986. Variation in temporal expression of overlapping baculovirus transcripts. Virus Res. 6:85-89.

FRIESEN, P.D., RICE, W.C., MILLER, D.W. and MILLER, L.K. 1986. Bidirectional transcription from a solo long terminal repeat of the retrotransposon Ted:

Symmetrical RNA start sites. Mol. Cell. Biol. 6:1599-1607.

67.041 CRISO097146 PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION

SCHULER M A; SLIGAR S G; Plant Biology; 809 South Wright Street, Champaign, **ILLINGIS** 61820.

Proj. No.: ILLR-85-1720 Project Type: CRG0 Agency ID: CRGO Period: O1 SEP 85 to O1 SEP 87

Objectives: Proj 8502328. The objectives of this projects are to characterize the levels of trans-cinnamic acid hydroxylase in pea seedlings. This P-450 monoxygenase, one of several documented plant cytochrome P-450 enzymes, is known to be induced and regulated by a wide variety of stimuli such as light, manganese, and herbicides as well as by synthetic plant auxins such as dichloro-phenoxyacetic acid (2,4-D). Further objectives include isolating and characterizing gene(s) coding for this cytochrome P-450 and establishing whether the P-450 trans-cinnamic acid hydroxylase gene is regulated at a transcriptional or translational level in light-induced plant tissue. Through this approach we hope to define, at the level of transcription and translation, the mechanisms for induction of a major plant cytochrome P-450.

Approach: To accomplish these goals we are developing a sensitive analytical method, using HPLC, to determine the actual induction levels of trans-cinnamic acid in response to light, 2,4-D and variety of other xenobiotics. In addition, we are characterizing genomic DNA sequences isolated from a pea recombinant library using the P-450 gene probes available from bacterial and mammalian systems.

67.042* CRISO033893 EXPRESSION OF EMBRYO-SPECIFIC GENES DURING IN VITRO EMBRYOGENESIS IN BRASSICA

CROUCH M C; Indiana University Foundation; Box 1847, Bloomington, **INDIANA** 47402.
Proj. No.: 8200500 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 82 to 28 FEB 86

Objectives: Proj. No. 8200500. We propose to study the expression of embryo-specific genes during the early stages of embryogenesis in Brassica napus L. Early biochemical events have been practically impossible to study in normal embryos of flowering plants because of inaccessibility of the embryo. Therefore, we propose to study adventive embryos that form in culture, unencumbered by endosperm and seed coat tissues, as a model for in vivo development. Using cloned cDNA probes, we plan to measure stroage protein mRNA levels during in vitro embryogenesis to determine how early and to what extent the embryo program is expressed. From this analysis we hope to learn about the relative importance of quantitative vs qualitative control of gene expression during plant embryo development. Our longterm goal is to understand what initiates and maintains the embryo program.

Approach: The development of a tissue culture system to study early embryo-specific gene expression is a necessary step towards this goal, and is the main objective of our proposal.

Progress: 82/10 to 84/09. Our goal was to develop an embryogenic tissue culture system for Brassica napus to study initiation of storage protein gene expression. We planned to use adventive embryogenesis as a model for in situ development since zygotic embryos are inaccessible early in development. First, we characterized cDNAs for the two major storage proteins, napin and cruciferin. Napin cDNA was sequenced and we showed that the protein is sythesized as a precursor and processed in an interesting way (Crouch et al., 1983). Cruciferin cDNA was also sequenced and compared with a related protein, legmin from peas (Simon and Crouch, in preparation). The comparison identified variable regions which may be good sites for future attempts to insert amino acids necessary for better nutritional qualities of the protein. We then used the cDNAs as probes to study levels of storage protein gene expression in somatic embryos. Attempts to initiate somatic embryos in suspension cultures were unsuccessful. However, we were able to obtain secondary embryogenesis from zygotic embroys cultured between 27 and 32 days after fertilization (Finkelstein and Crouch, 1984; Simon and Crouch, in preparation). These secondary embryos accumulated storage protein mRNAs to levels characteristic of the stage of the primary embryos, then proliferated embryos on their own surfaces: we established a permanently embryogenic tissue culture system which cycled between 0- and 30-day embryo stages.

Publications: 82/10 to 84/09

FINKELSTEIN, R.R. and CROUCH, M.L. 1984.
Precociously germinating rapeseed embryos
retain characteristics of embryogeny.
Planta 162:125-131.

CROUCH, M.L., TENBARGE, K.M., SIMON, A.E. and FERL, R. 1983. cDNA clones for Brassica napus seed storage proteins: evidence from nucleotide sequence analysis that both subunits of napin are cleaved from a precursor polypeptide.

CROUCH, M.L., TENBARGE, K., SIMON, A., FINKELSTEIN, R., SCOFIELD, S., and SOLBERG, L. 1984. Storage protein mRNA levels can be regulated by abscisic acid in Brassica embryos. Im "Molecular Form and Function of the Plant CROUCH, M.L. 1983. The use of macromolecular markers to study plant embryo development in vitro. In vitro. 19:238.

67.043 ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTDNA

SINCLAIR J H; Biology; Indiana University, Bloomington, **INDIANA** 47401.

Proj. No.: 8100376 Project Type: CRG0 Agency ID: CRG0 Period: 01 SEP 81 to 31 AUG 86 Objectives: The large (300-400 Kbp) mitochondrial genome of Zea mays has been restriction digested and a pBR322 clone library prepared. A map is being prepared. Individual fragments will be physically and functionally characterized. Particular attention will be given to high AT and high GC regions, repetitions segments, palindromes and inverted segments. This will help explain the complex arrangement of the DNA and help explain why higher plant mtDNA is over 20 times the size of animal mtDNA. It may also explain the rearrangement undergone in cytoplasmic male sterility. Some segments will be sequenced.

Approach: Conventional methods for recombinant DNA work, denaturation analysis, DNA/DNA hybridization, heteroduplex analysis, buoyant density centrifugation, sequencing, etc., will be utilized.

Progress: 85/09 to 86/08. Progress toward understanding of the genetic arrangement of Zea Mays mtDNA during the period covered by this grant has been significant. Unfortunately most all of it was made in other laboratories with other support. My laboratory did do a very large amount of work preliminary to generating a restriction map and a gene map of this genome. All of the DNA segments of the large mtDNA genome was collected in clone banks, fragments purified, identified regarding their location on a restriction pattern, subrestriction maps made and a large body of cross-fragment hybridation was done to to generate a fragment order of map. After much work was done, we discovered that the genome was at an upper limit of size for this strategy of mapping and the added complication of multiple repeated units made the strategy of working with individual restriction fragments unacceptably cumbersome and slow. Fortunately for higher plant genome workers, David Lonsdale had generated a clone bank of very large fragments in cosmid vectors, permitting him to bypass our problems so he generated the map we were working toward before wwe could. We redirected our efforts toward working to locate individual genes and sequence them. The location of the rRNA genes were determined and published and the sequence of the histidyl tRNA (which was transferred to the mitochondrion from the chloroplast) was determined and reported.

Publications: 85/09 to 86/08

IAMS, K.P. and SINCLAIR, J.H. 1982. Mapping the mitochondrial DNA of Zea Mays: Ribosomal Gene Location. Proc. Natl. Acad. Sci. USA 79:5926-5929.

IAMS, K.P. 1983. Ph.D. Thesis, Dept of Biology, Indiana University. Partial Characterization of the Mitochondrial DNA of Zea Mays: Ribosomal RNA, Transfer RNA and Rearrangements in CMS Strains.

IAMS, K.P., HECKMAN, J.E. and SINCLAIR, J.H. 1985. Sequence of histidyl tRNA, Present as a Chloroplast Insert in mtDNA of Zea Mays. Plant Molecular Biology 4:225-232.

67.044 CRISO011497 STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS

AXELROD B; Biochemistry; Purdue University, West Lafayette, INDIANA 47907.

Proj. No.: INDO53049 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 89

Objectives: To determine the amino acid sequences of the lipoxygenase isozymes of soybeans, in order to obtain the three dimensional structures, in collaboration with cooperating protein crystallographers; and to identify and compare the iron binding and active sites of the lipoxygenase isozymes.

Approach: Messenger-RNA corresponding to the three isozymes of lipoxygenase L-1, L-2, and L-3 will be obtained from immature seeds by standard techniques. Isolation and identification will be aided by use of specific antibodies. The corresponding c-DNAs will be cloned using a recombination technique designed to yield full length d-DNA sequences. Sequences will be done by the Sanger procedure. Amino acid sequencing will be carried out on pure isozymes to the extent needed for alignment and interpretation of the nucleotide sequences. Attempts will be made to identify the iron-binding sites which are presumably similar in all of the isozymes. This effort will be guided by knowledge of the amino acid sequences and the tertiary structure from the x-ray studies. In addition current literature on other non-heme, non-iron sulfur iron dioxygenases may also help suggest possible binding sites. Lipoxygenase in which the normal iron is replaced with radioisotopic iron will be subjected to controlled degradation in an effort to obtain a smaller fragment amenable to examination.

Progress: 87/10 to 88/09. We previously solved the primary sequences of soybean lipoxygenase-1 and -2. Examination of these structures based on our previous physico-chemical studies as well as on the present state of knowledge of iron-containing proteins has permitted us to identify a putative iron-binding region and active site in these enzymes. The importance of this region, consisting of a 40-amino acid sequence rich in histidine, is greatly strengthened by a recent report that this sequence is significantly conserved in a lipoxygenase from a highly divergent species, namely, human white blood cells. We are presently engaged in carrying out site-specific mutations in soybean lipoxygenase-1 in order to evaluate the essentiality of the components of this sequence. The same approach will be applied to other sectors of the molecule in order to understand their roles in the overall behavior of the enzyme. The information to be gained may aid in the ultimate control of the problem of off-flavors which beset the processing of soybeans for human food. Of perhaps greater importance, is that the knowledge gained may contribute to the control of the animal lipoxygenase, especially 5'-lipoxygenase, a progenitor of the leukotrienes, which play a major role in serious human immunological disorders.

Publications: 87/10 to 88/09
SHIBATA, D., STECZKO, J., DIXON, J. E.,
ANDREWS, P. C., HERMODSON, M. A., and
AXELROD, B. (1988). Primary structure of
soybean lipoxygenase L-2. J. Biol. Chem.
263:6816.

67.045 CRISO034239 REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNDS IN HIGHER PLANTS

HERRMANN K M; Biochemistry; Purdue University, West Lafayette, INDIANA 47907.

Proj. No.: INDO53047G Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 84 to 30 SEP 87

Objectives: PROJ 8400608. The long term goal of this project is to identify specific DNA sequences that function as control elements in the expression of genes encoding key enzymes of plant metabolism. The primary focus will be on the DNA region(s) preceding the coding sequence(s) for 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase from carrot (Daucus carota).

Approach: Isolation of sufficiently pure DAHP synthase from carrot to determine the amino terminal sequence of this enzyme; chemical synthesis of an oligonucleotide that encodes a suitable portion of the amino terminal sequence of carrot DAHP synthase; use of this oligonucleotide to probe a carrot genome bank for DNA fragments encoding DAHP synthase; cloning of such fragments into a bacterial vector; DNA sequence analysis to identify the coding sequence for carrot DAHP synthase and the 5'-flanking sequence which is expected to be the target site for macromolecules that affect the expression of this gene.

Progress: 87/01 to 87/12. Roots of carrots (Daucus carota) contain three activities of 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase, the enzyme that catalyses the first step of the shikimate pathway. The three activities, enzymes I, II, and III, are separated by chromatography on phosphocellulose. Enzyme III, purified to electrophoretic homogeneity, has a native molecular weight of 103,000 and consists of two identical subunits of 53,000 daltons each. Double reciprocal plots of reaction velocity versus substrate concentration yield K(M) values of 0.03 and 0.073 millimolar for P-enolpyruvate and erythrose-4-P, respectively. Both products, DAHP and orthophosphate, inhibit the enzyme. Enzyme III is a hysteretic enzyme that is activated by physiological concentrations of L-tryptophan and Mn of which also partially eliminate the hysteretic lag. Feedback activation of carrot DAHP synthase by tryptophan is interpreted to be an early regulatory signal for polyphenol biosynthesis. The three carrot DAHP synthase isoenzymes share antigenic determinants. DAHP synthase was also purified to electrophoretic homogeneity from tubers of Solanum tuberosum L. cv. Superior. The enzyme is also a dimer, with a native molecular weight of 110,000. The enzyme also appears to be hysteretic. The enzyme activity is stiumulated by Mn

tryptophan. Chromatofocussing resolves two forms of the enzyme with isoelectric points of 7.8 and 8.4, respectively.

Publications: 87/01 to 87/12

- SUZICH, J.A., DEAN, J.F.D. and HERRMANN, K.M. 1985. 3-Deoxy-D-arabino-heptulosonate 7-phosphate synthase from carrot root (Daucus carota) is a hysteretic enzyme. Plant Physiol. 79:765-770.
- DYER, W.E., PINTO, J.E.B.P. and HERRMANN, K.M. 1986. Glyphosate induced activation of 3-deoxy-D-arabino-heptulosonate 7-phospate (DAHP) synthase in potato cells. J. Cell. Biochem. 10C:44.
- PINTO, J.E.B.P., SUZICH, J.A. and HERRMANN, K.M. 1986. 3-Deoxy-D-arabino-heptulosonate 7-phosphate synthase from potato tuber (Solanum tuberosum L.) Plant Physiol. 82:1040-1044.

67.046 OO10462 STRUCTURE OF PHOTOSYNTHETIC MEMBRANES

KROGMANN D W; Biochemistry; Purdue University, West Lafayette, INDIANA 47907.
Proj. No.: INDO53043 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 83 to 30 SEP 89

Objectives: The objective of this work is to understand in precise chemical terms how plants convert radiant energy from the sun into the stable chemical compounds of newly grown plant material. The photosynthetic process occurs in membranes contained in the chloroplast. The membrane must be taken apart, the individual components purified and each component must be characterized in terms of the structure, its function and its interaction with neighboring molecules in the membrane.

Approach: The approach to this problem relies on the application of biochemical techniques to dissolve the membrane and to purify each of its components. When a protein has been purified, its amino acid sequence is determined and an effort is made to crystallize it in a form suitable for X-ray diffraction analysis by other laboraties. We have and will continue to apply techniques of nuclear magnetic resonance, electron spin resonance, optical spectroscopy, and enzymatic and chemical probing to describe the interactions among components.

Progress: 87/10 to 88/09. The goal of this research is to understand photosynthesis in terms of the structures of individual proteins which catalyze essential reactions in energy conversion by green plants. Substantial progress has been made in defining the sites of interaction when an electron is passed from cytochrome f to plastocyanin during photosynthetic electron transport. The two purified proteins were allowed to interact, covalently cross linked, digested to small fragments, the fragments purified and sequenced and the points of contact between the two defined by the cross linked residues. In another project a new zeaxanthin containing carotenoid protein complex was isolated from Anacystis nidulans and partially characterized. We are examining the biological role of this

protein as a protectant against photodestruction or as an antenna for energy collection.

Publications: 87/10 to 88/09

- YAMAMOTO, Y., NAKAYAMO, S., COHN, C. L., and KROGMANN, D. W. (1987). Highly efficient purification of the 33-, 24-, and 18-kDA proteins in spinach photosystem II by butanol/water phase partitioning and high performance liquid chromatograph.
- MEYER, T. E., CUSANOVICH, M. A., KROGMANN, D. W., BARTSCH, R. C., and TOLLIN, G. (1987). Kinetics of reduction by free flavin semiquinones of algal cytochromes and plastocyanin. Arch. Biochem. Biophys. 258:307.
- DIVERSE-PIERLUISSI, M. and KROGMANN, D. W. (1988). A zeaxanthin protein from Anacystis nidulans. Biochim. Biophys. Acta 933:372-377.
- OVERHOLT, C. (1987). Studies on cytochromes c(subscript 553), c(subscript 550), and ferredoxins I and II from Microcystis aeruginosa. M.S. thesis. Purdue Univ., W. Lafayette, IN. 50 pp.
- WHITAKER, R. A. (1987). Genes of photosynthetic catalysts in cyanobacteria. M.S.
- thesis. Purdue Univ., W. Lafayette, IN. 36 pp.
- DIVERSE-PIERLUISSI, M. (1987). A carotenoid protein from Anacystis nidulans and synthesis of cytochrome c(subscript 550) in cyanobacteria. M.S. thesis. Purdue Univ., W. Lafayette, IN. 47 pp.
- HARPER, C. F. (1988). A comparative study of proteins isolated from Microcystis aeruginosa. M.S. thesis. Purdue Univ., W. Lafayette, IN. 59 pp.

67.047 CRISOO14698 PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES

JACKSON A 0; Botany & Plant Pathology; Purdue University, West Lafayette, INDIANA 47907.

Proj. No.: INDO55040 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 84 to 31 DEC 85

Objectives: To develop an understanding of infectious processes of plant viruses, and events leading to development of disease syndromes in infected plants. Probe biochemical events which occur in plants during infection with plant viruses. To identify viral specific products and investigate the roles of the products in viral replication. To diagnose and characterize viruses causing new diseases of maize and small grains.

Approach: Replication of RNA viruses with RNA genomes will be investigated. Viral specific DNA clones will be used as probes to detect viral RNAs in infected plants. The clones will also be used for nucleic acid sequencing in affinity chromotography to isolate viral messenger RNAs from infected plants. The messenger RNAs will be translated in in vitro protein synthesis systems to identify the viral specific polypeptides. The response of plants to virus infection will be measured by comparing RNA and protein synthesis of healthy

and virus infected plants or protoplasts. Electron microscopy, serology and virus transmission experiments will be conducted to diagnose maize and small grain diseases caused by viruses in Indiana.

Progress: 85/01 to 85/12. We have found that tobacco infected with the plant rhabdovirus, sonchus yellow net virus (SYNV), contains short, 139 to 144 nucleotide long transcripts complementary to the 3' terminus of the negative-stranded genomic RNA. These transcripts are similar to the leader RNAs associated with several animal rhabdovirus infections in that they are coded by the same region of the genome, but the SYNV transcripts are nearly three times longer than the animal rhabdovirus leader RNAs. The SYNV leader RNAs differ markedly in sequence from the leader RNAs associated with strains of vesicular stomatitis virus (VSV) and rabies virus. although the first 30 nucleotides of all three transcripts are rich in adenine residues. The nucleotide sequence determined directly from from SYNV RNA and from recombinant DNA clones derived from SYNV RNA reveals a possible start site for the N protein gene that is located 147 nucleotides from the 3' end of genomic RNA. The sequence (UUGU) at this site is identical to the first four nucleotides of the N protein genes of animal rhabdoviruses. In SYNV, the first AUG codon in the putative N protein gene is located 57 nucleotides downstream, at positions 203-205, and is followed by an open reading frame for the remainder of the 1020 nucleotides determined in these experiments.

Publications: 85/01 to 85/12

ZUIDEMA, D., HEATON, L.A., HANUA, R., and JACKSON, A.O. 1985. Detection and sequence of plus-stranded leader RNA of sonchus yellow net virus, a plant rhabdovirus. Submitted to Proc. National Acad. Sci. (USA), Purdue Ag. Exp. Sta.

ZUIDEMA, D., HEATON, L.A. and JACKSON, A.O. 1985. Sequence analysis at the 3' terminus of sonchus yellow net virus. 1st International Congress of Plant Molecular Biology Abstracts p. 30.

Biology Abstracts p. 30.
HEATON, L.A., ZUIDEMA, D. and JACKSON, A.O.
1985. Sequence analysis at the 3' terminus
of the genome RNA of sonchus yellow net
virus. Phytopathology 75:1335.

67.048 CRISO130500 CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS

BRESSAN R; Horticulture; Purdue University, West Lafayette, INDIANA 47907.
Proj. No.: INDO65058-G Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 86 to 31 AUG 89

Objectives: Project 8601632. Cloning and characterization of DNA sequences (stress protein gene(s)) whose expression is highly correlated with the tolerance of plant cells to salt stress. Determine the natural regulation of expression of this gene(s).

Approach: The goal of the experiments outlined in this proposal is to isolate gene(s) involved in the salt and water stress tolerance of higher plants and to study their regulation of expression during salt stress. This goal will be fulfilled by cloning particular DNA sequences which encode a specific protein whose synthesis is specifically induced and enhanced in stress tolerant cell lines (39) (stress protein). We will then use these sequences as probes to study the organization and expression of such genes and how such organizatioon and expression is involved in salt stress tolerance in plant cells.

Progress: 87/01 to 87/12. Cultured tobacco (Nicotiana tabacum var Wisconsin 38) cells adapted to grow under osmotic stress synthesize and accumulate a 26 kilodalton protein (osmotin) which can constitute as much as 12% of total cellular protein. In cells adapted to NaCl, osmotin occurs in two forms: an aqueous soluble form (osmotin-I) and a detergent soluble form (osmotin-II) in the approximate ratio of 2:3. Osmotin-I has been purified to 90% electrophoretic homogeneity, and osmotin-II has been purified to 90% electrophoretic homogeneity. The N-terminal amino acid sequences of osmotins I and II are identical through position 22. Osmotin-II appears to be much more resistant to proteolysis than osmotin-I. However, it cross-reacts with polyclonal antibodies raised in rabbits against osmotin-I. Osmotin strongly resembles the sweet protein thaumatin in its molecular weight, amino acid composition. N-terminal sequence. and the presence of a single peptide on the precursor protein. Thaumatin does not cross-react with antiosmotin. An osmotin solution could not be detected as sweet at a concentration at least 100 times that of thaumatin which could be detected as sweet. Immunocytochemical detection of osmotin revealed that osmotin is concentrated in dense inclusion bodies within the vacuole. Although antiosmotin did not label organelles, cell walls, or membranes, osmotin appeared sparsely distributed in the cytoplasm.

Publications: 87/01 to 87/12

LAROSA, P.C., HASEGAWA, P.M., RHODES, D. and BRESSAN, R.A. Osmotic adjustment stimulated by abscisic acid and its involvement in adaptation to NaCl in cultured tobacco cells. Plant Physiology. In Press.

BINZEL, M., HASEGAWA, P.M., RHODES, D., HANDA, S. and BRESSAN, R.A. Solutes involved in the osmotic adjustment of tobacco cells adapted to NaCl. Plant Physiology. In Press.

SINGH, N.K., HANDA, A.K., HASEGAWA, P.M., BUCKEL, S., HERMODSON, M.A., PFANLOECH, E., BRACKER, C., REGNIER, F.E. and BRESSAN, R.A.

67.049 CELLULAR METABOLISM IN PLANTS

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0032385

CHERRY J H; Horticulture; Purdue University, West Lafayette, INDIANA 47907.

Proj. No.: INDO65019 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: Explore the subject of heat shock and heat shock protein (HSP) synthesis to develop the means of determining heat resistance of plants in a given environment. Determine the mechanisms involved in the regulation of unsaturated fatty acid synthesis in developing soybean seed.

Approach: Study the biosynthesis of proteins in plant cells under heat stress conditions. Study various enzymes that regulate the synthesis of fatty acids, in general, and specifically unsaturated fatty acids.

Progress: 87/10 to 88/09. Last year, we reported that two heat shock proteins are expressed in high quantity in cowpea (Vigna unguiculata) cell suspensions adapted to 38(degree)C. These HSPs have apparent molecular weights of 70 and 80 kDa. In addition to these proteins, we have identified at least two others which. are expressed abundantly in heat-adapted cells but not in cells maintained at 26(degree)C. A protein, with an apparent molecular weight of 22 kDa, is also synthesized under heat shock conditions and produces two distinct spots with very close pI's on two dimensional polyacrylamide gel electrophoresis. The second protein, with an apparent molecular weight of 31 kDa, does not seem to be a major heat shock protein. Both are acidic proteins, are visible on Coomassie-stained gels as well as fluorograms of (superscript 3)H- labelled cells, and comprise a substantial portion of the total protein staining. We have begun purification procedures on these proteins to determine their roles in the adaptation of cells to high levels of heat. We plan to isolate messenger RNAs and measure the in vitro translated products with an intent to learn more about the genetic expression of the proteins under thermoadaptation.

Publications: 87/10 to 88/09

86(4):50.

BARKLEY, G.M. and CHERRY, J.H. (1988). Joseph C. Arthur (1850-1942): Contributions to the development of plant physiology in the United States. Plant Physiol. 86(4):3.

HARRIMAN, R.W., CHERRY, J.H., and HANDA, A.K. (1988). Purification and characterization of tomato fruit pectinmethyl-esterase (PME). Plant Physiol. 86(4):44.

MAYER, R.R. and CHERRY, J.H. (1988). Identification and partial purification of proteins involved in adaptation of cowpea

cells to high temperatures. Plant Physiol.

67.050 CRISO133494
IDENTIFICATION OF REGULATORY SEGMENTS IN A
WOUND-INDUCIBLE INHIBITOR II GENE.

THORNBURG R W; Biochemistry & Biophysics; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: IOW02859 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 87 to 30 SEP 90

Objectives: Prepare a nested set of promoter deletions from the Inhibitor II promoter and characterize these deletions by restruction mapping and nucleotide sequencing. Move the deletions into a pair of binary vectors containing CAT gene and transform whole plants. Quantitate wound-inducible CAT activity and correlate this activity with the structure of the Inhibitor II Promoter regions. Exchange the native Inhibitor II terminator for several other terminators to examine the effect of alternative termination sequences on the wound-inducible induction of the Protennase Inhibitor II genes. PROJ. 8701110.

Approach: A Bal 31 treatment of the cloned Inhibitor II CAT chimeric gene will be used to generate a set of nested deletions of the chimeric gene. These promoter deletions will be sequenced by Maxim and Gilbert modification methods.

Progress: 88/01 to 88/12. We ave isolated an additional proteinase inhibitor II gene from a potato genomic library. This gene is located on a 17 kb EcoRI fragment. We have subcloned a 3.6kb fragment which contains the entire pin2 gene. At the same time which we have been preparing clones for sequencing, we have prepared a series of clones which represent promoter deletions. These range in size from 2.6 kb to less than 400 bp. We are currently inserting these plasmid deletions into a plant transformation vector, pBI121 which contains the beta-glucuronidase marker gene. We will use this promoter deletion series to transform both tobacco and potato plants. With these plant transformation vectors we will be able to address the original goal of the proposal which was to determine those regions in the 5' end of the gene which are responsible for woundinduction of proteins in transgenic plants.

Publications: 88/01 to 88/12
No publications reported this period.

67.051 CRISO090502 GENETICS OF FUNGAL PLANT PATHOGENS

BRONSON C R; Plant Pathology; Iowa State
University, Ames, **IOWA** 50011.
Proj. No.: IOW02632 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 91

Objectives: To develop the genetic system of Cochliobolus heterostrophus for use in research on mechanisms of fungal pathogenesis to plants.

Approach: a) develop an RFLP may using random Cochliobolus DNA and cloned genes from Cochliobolus and related organisms; b) characterize factors (chromosome rearrangements

and Spore-killer loci) affecting fertility; c) improve mutagenesis protocols; d) collect mutants involved in pathogenicity.

Progress: 88/01 to 88/12. The long term goal of this project is to understand how fungi cause plant disease. Cochliobolus heterostrophus, the causal agent of southern leaf blight of maize, has been chosen as a model organism. Our immediate goal is to simplify the cloning of genes in C. heterostrophus involved in pathogenesis by developing a restriction fragment length polymorphism (RFLP) map of its genome. The map is now nearing completion. Parental strains were selected by screening field isolates for RFLPs relative to a standard laboratory strain. These strains were crossed and their progeny collected. Probes for detection of RFLPs were prepared from random Cochliobolus nuclear DNA and used to determine linkage relationships in the progeny. At the time of this writing, 95 markers (91 RFLP and 4 phenotypic) have been analyzed. Of these, 76 have shown significant linkage to at least one other marker, indicating coverage of about 80% of the genome. The map length is roughly 815 centiMorgans, suggesting that the total length may be about 10 Morgans, similar to that of Neurospora crassa. We are now in the process of refining the map by the analysis of additional markers and progeny. We have also been successful in the separation of whole Cochliobolus chromosomes and are using this technique for confirmation of linkage relationships.

Publications: 88/01 to 88/12
BRONSON, C. R. (1988). Ascospore abortion in
 crosses of Cochliobolus heterostrophus
 heterozygous for the virulence locus Toxl.
 Genome 30:12-18.

67.052 CRISO077755 THE PHYTOPATHOGENIC BACTERIA

CHATTERJEE A K; Plant Pathology; Kansas State University, Manhattan, **KANSAS** 66506.

Proj. No.: KANOO082 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 89

Objectives: Our long term research program has two interrelated general objectives: (i) to learn the properties of bacterial pathogenic determinants; and (ii) to elucidate the mechanisms underlying bacterial pathogenicity towards higher plants.

Approach: For a meaningful development of this program, we intend to apply technologies in various disciplines such as molecular biology, ecology, recombinantional genetics, metabolism, and plasmid biology. This concerted multidisciplinal approach will provide knowledge that relates to the general aims of the program thus filling the void which prevails in our understanding of the biology of these organisms.

Progress: 88/01 to 88/12. The carotovoricin (CTV) and pectin lyase (PNL) phenotypes in the bacterium Erwinia carotovora subsp. carotovora (Ecc) were shown to be recA-dependent and

expressed in potato tuber tissue. Tn5 insertion mutants of Ecc71 defective either in PNL or CTV $\,$ contained a single copy of the transposon located in different regions of the genome. Our data suggest that ctv and pnl are independent transcriptional units inducible by a common signal. Tn5 insertion mutants of Pseudomonas syringae pv. syringae (Pss) were used to study the organization of genes for syringotoxin (ST) production. Southern blot data indicated that the loss of toxin production was associated with Tn5 insertions into chromosomal EcoR1 fragments of about 10.5, 17.8, and 19.3 Kb and that the 10.5 and 17.8 Kb fragments were contiguous. By comparing the protein profile of Tn5 insertion St mutants with those of the ST parent, we tentatively associated two proteins (465 and 430 Kd) with ST production. We also detected strong homology between ST associated sequences and genomic DNA of Pss strains that produce syringomycin, indicating relatedness between peptide toxin synthesizing systems in these bacterial strains. The Tn5 fragments were cloned and characterized by restriction analysis. Marker exchange with the cloned DNAs established linkage between the Tn5 insertions and the ST phenotype. A Tox derivative of a highly pathogenic ST-producing strain, constructed by marker exchange recombination, was severely attenuated in plant virulence.

Publications: 88/01 to 88/12
WILLIS, J.W., ENGWALL, J.K. and CHATTERJEE,
A.K. 1987. Cloning of genes for Erwinia carotovora subsp. carotovora pectolytic enzymes and further characterization of the polygalacturonases. Phytopathology 77:1199-1205.

67.053 CRISO130099 ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELOIDOGYNE INCOGNITA

WHITE F F; Plant Pathology; Kansas State
University, Manhattan, **KANSAS** 66506.
Proj. No.: KAN09453 Project Type: CRG0
Agency ID: CRG0 Period: 15 SEP 86 to 31 MAR 89

Objectives: PROJECT 8601782. The goal of this proposal is to isolate a gene which is tightly linked to a disease resistance locus in tomato. The Mi locus (nematode resistance) in tomato is tightly linked to the isozyme acid phosphatase-1 (APS-1). By isolating APS-1, we propose to clone the APS-1 gene and use the gene as the starting point for "chromosome walking" to the Mi locus.

Approach: The approach will involve isolating the APS-1 enzyme from nematode resistant tomato plants using standard protein isolation technique. The protein will be characterized and the N-terminus amino acid sequence determined. The sequence will be used to generate a oligonucleotide probe for screening a recombinant DNA library form tomato.

Progress: 86/09 to 88/09. The objectives of the project were to devise a purification scheme for the APS-1 (acid phosphatase) protein of tomato; purify sufficient quantities of the protein for physical characterization; and

develop a practical test for the transfer of nematode resistance. Toward these goals, the following was accomplished: A purification scheme was developed during the 1986-1987 period. Fresh Tomato seedlings were homogenized in extraction buffer (0.01 M histidine, pH6.0, 40 mM - mercaptoethanol, 0.1% PMSF) and centrifuged. A subsequent 95% ammonium sulfate precipitate was recovered from the supernatant. This material was dialyzed and fractionated sequentially on CM52 (carboxymethyl cellulose), DEAE 52 (anion exchange), chromatofocussing (pH 6.0 to pH 4.0), and Concanavaline-A affinity chromatography. The extensive purification procedure yielded in most preparations a single band of apparent molecular size of 28 kilodaltons in a denaturing polyacrylamide gel. A test system for eventual testing of nematode susceptibility was initiated. The root-inducing ability of Agrobacterium rhizogenes was exploited. The project changed focus at this point in order to better characterize the role of the individual genes in the root-inducing (Ri) plasmid of A. rhizogenes. The results indicated that in tobacco and Kalanchoe the rolB gene primarily controlled the rooting response. Inclusion of rolC functions in promoting root growth.

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Publications: 86/09 to 88/09 NO PUBLICATIONS REPORTED THIS PERIOD.

67.054 CRISO032465 VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS

SHAW J G; Plant Pathology; University of Kentucky, Lexington, **KENTUCKY** 40506. Proj. No.: KY00856 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 90

Objectives: Determination of the strategy of expression of the potyviral genome in infected cells. Insertion of DNA, corresponding to the entire genome of tobacco vein mottling virus (TVMV, a potyvirus), in a transcription vector. Determination of the effects of selected alterations of the nucleotide sequence of TVMV cDNA on the in vivo activity of transcripts of the cloned DNA.

Approach: Tobacco protoplasts will be electroporated with TVMV RNA; S methionine labelled polypeptide will be immunoprecipitated and the time course of potyviral protein production will be determined. A double stranded cDNA containing the complete TVMV RNA nucleotide sequence will be constructed and transcribed in vitro. Full length TVMV transcripts will be assayed for infectivity and for in vitro messenger activity. Site specific mutagenesis will be done, followed by in vitro transcription and assay for biological activity.

Progress: 88/01 to 88/12. Potential protease functions encoded by tobacco vein mottling virus (TVMV) RNA were investigated. To explore the potential role of the NIa nuclear inclusion protein in proteolytic processing, a modified in vitro "hybrid-arrested translation" of TVMV RNA was utilized as well as the translation of

in vitro transcripts of cloned cDNA encoding the TVMV NIa protein. When viral RNA was first hybridized to DNA probes complementary to the NIa cistron, and then treated with RNase H prior to translation, a 48-kDa processing product, immunologically identified as the NIa protein, was abolished. In its place was observed a series of larger polypeptides, immunologically identified as fusion products of the cylindrical inclusion (CI) and NIa cistrons. None of these DNA probes affected the cleavage between the helper component (HC) and 42K proteins. Transcripts of cloned cDNA representing the NIa protein and flanking sequences produced translation products, not of a large fusion polyprotein, but of a fully processed NIa polypeptide. Transcripts lacking coding sequences for the hypothesized NIa protease active site remained unprocessed. These data indicate that NIa functions as a protease for the maturation of some but not all TVMV proteins. Processing of HC, an event apparently not mediated by the NIa protease, was examined. Hybrid-arrested translation reactions which prevented synthesis of the 42K protein displayed correct processing.

Publications: 88/01 to 88/12

HELLMANN, G. M., SHAW, J. G. and RHOADS, R. E. (1988). In vitro analysis of tobacco vein mottling virus NIa cistron: evidence for a virus-encoded protease. Virology 163:554-562.

SHAHABUDDIN, M., SHAW, J. G. and RHOADS, R. E. (1988). Mapping of the tobacco vein mottling virus VPg cistron. Virology 163:635-637.

DOMIER, L. L., FRANKLIN, K. M., HUNT, A. G., RHOADS, R. E. and SHAW, J. G.

Infectious in vitro transcripts from cloned cDNA of the potyvirus, tobacco vein mottling virus. Proc. Natl. Acad. Sci. USA. In press.

PIRONE, T. P. and SHAW, J. G. (1988). Tobacco vein mottling virus. AAB Descriptions of Plant Viruses, No. 325, 4 pp.

67.055* CRISO131900 DEFINING AND MAPPING THE GENES OF CAULIMOVIRUSES

SHEPHERD R J; Plant Pathology; University of Kentucky, Lexington, **KENTUCKY** 40506.

Proj. No.: KY00872 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 90

Objectives: To study the functions and role in pathogenesis of the genes of cauliflower mosaic virus (CaMV), a small DNA virus of plants.

Approach: The virus genome has been completely sequenced and the specific location of each tentative gene is precisely known and a catalog of restriction sites is known for use in restructuring the DNA. The DNA can be cloned in bacteria and is infectious to plants when cut free of the cloning vector. Hence, with recombinant DNA methods it is easy to manipulate the virus genetically. In addition single virus genes are being inserted in the plant chromosome using the Ti plasmid-Agrobacterium system. Various promoters

of different strength will be coupled to CaMV gene V1 and integrated into plant chromosomes to relate magnitude of disease with gene V1 expression. In addition the cytological effects and virus susceptibility of gene V1 transformed plants will be determined by conventional methods.

Progress: 88/01 to 88/12. An integrative transformation strategy is being used to evaluate the level and character of disease induced by caulimovirus gene VI expression in host plants. We have cone Ti-plasmid-Agrobacterium transformations of gene VI of cauliflower mosaic (CaMV) and figwort mosaic (FMV) viruses in Nicotiana tabacum or Datura innoxia. Transformation with gene VI of either virus induces a chlorosis and mottling-type disease similar in appearance to virus infections. Symptom development is closely associated with high level expression of the gene VI protein (P62). However, in most cases where symptoms develop (CaMV or FMV in N. tabacum or CaMV in D. innoxia) the plant is not a systemic host of the virus in question. For this reason we have developed a procedure for regenerating Nicotiana edwardsonii which is a systemic host for all three viruses (CaMV, FMV and peanut chlorotic streak viruses). Consequently, we are now in a position to determine if gene VI expression will perturb natural systemic hosts to cause disease. Gene VI RNA transcripts are being electroporated into protoplasts followed by treatment of extracts with chemical cross linkers followed by Western blotting analyses for P62 in an effort to discover if this protein becomes closely associated with a host protein in order to carry out its function. This approach has not yielded positive results yet.

Publications: 88/01 to 88/12 SHEPHERD, R.J., GOLDBERG, K-B., KIERNAN, J., GOWDA, S., SCHOELZ, J., YOUNG, M. and RICHINS, R. (1988). Genomic changes during host adaption by caulimoviruses. In Physiology and Biochemistry of Plant-Microbial Interactions. SHEPHERD, R.J. (1988). The biochemistry of DNA plant viruses. In The Biochemistry of Plants, a Comprehensive Treatise. Vol. 6. Proteins and Nucleic Acids. P. Stumpf and E.E. Conn, Eds. Academic Press, pp. 565-618.

67.056* CRISO141166 MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES

MATTHEWS B F; Agricultural Research Service; Beltsville Agr Res Center, Beltsville, MARYLAND 20705.

Proj. No.: 1275-22000-003-00D

Project Type: INHOUSE Agency ID: ARS Period: O1 APR 86 to 25 JUN 87

Objectives: Develop technologies for bypassing traditional sexual breeding barriers andmaternal inheritance to create variation and unique gene combinations for improving productivity of economically important crop plants, such as wheat, rice and soybean and to

map and study the regulation of important genes in these crop systems.

Approach: 1) Transfer portions of chloroplast, mitochondrial and nuclear genomes between species, 2) develop a map and determine the structural organization of mitochondrial genome in the recipient parent and hybrid cell lines, 3) determine the effects of chloroplast transfer on nuclear-chloroplast interaction by monitoring production & regulation of key enzymes (aspartokinase, homoserine dehydrogenases and dihydrodipicolinic acid synthase) involved in synthesis of essential and nutritionally important amino acids, lysine, threonine and methionine from aspartate, and 4) clone nuclear genes encoding these enzymes. Enzyme activities, located mainly inthe chloroplast, will be measured and characterized. Thus, these enzymes will be examined at the gene, mRNA and protein levels to understand mecha- nisms regulating this typical plant biosynthetic pathway. BELTSVILLE, MD; BG 010, RM 9 & 10; BL-1; 12/05/85. B. Matthew, C. Cohen, L. DeBonte.

Progress: 87/01 to 87/06. The cox II gene from the mitochondrion of carrot has been isolated and mapped. A complex intron is present. Portions of the cox II gene and intron have been sequenced to confirm these observations. Research benefited other scientists conducting research in genetic engineering.

Publications: 87/01 to 87/06 NO PUBLICATIONS REPORTED THIS PERIOD.

67.057 CRISO096956 THE REPLICATION OF CHLOROPLAST DNA IN CHLAMYDOMONAS REINHARDII

WU M C; Biological Sciences; University of Md 5401 Wilkens Ave, Catonsville, MARYLAND 21228. Proj. No.: MDR-8500197 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 85 to 31 AUG 86

Objectives: Proj 8500197. Comparative sequencing analyses of the Chloroplast (Ct)DNA replication origins isolated from two different species of Chlamydomonas. Since two back-to-back promoters are detected within one cloned replication origin by using the promoter cloning vectors, pkoI and pko TWI. Detail analyses of the transcription start sites will be carried out. Whether any transcript is used as a primer for CtDNA replication will be analyzed.

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Approach: Nucleotide sequence will be determined by using the Maxim-Gilbert technique and the dideoxy chaintermination technique. DNA sequence comparison will be analyzed by using a dot-matrix computer program. The transcription start site will be determined by using the S-1 mapping technique. The newly synthesized DNA-RNA hybrid molecules will be isolated from an in vitro DNA replication system, the RNA-DNA junction will be determined by treatment with RNase to remove RNA moiety from the hybrid molecules.

Progress: 85/09 to 86/08. A chloroplast DNA replication origin, Ori A, isolated from two different species of Chlamydomonas had been cloned and sequenced. The function of one cloned Ori A has been verified and analyzed in an in vitro DNA replication system constructed from partially purified algal proteins. Initiation site for DNA replication in this in vitro system has been mapped. Sequence comparison shows that most conserved sequences between these two cloned origins are located within an open reading frame (ORF). Amino acid sequences of these two origin ORFs are highly conserved. In one origin, 8 copies of a tandomly repeated sequence have been located near the ORF. Functional significance of these repeated sequence will be analyzed. The transcriptional activities of the ORF & its flanking region has been analyzed. Two back to back procaryotic promoter have been mapped within the ORF. Several transcription initiation sites and transcription termination sites have been mapped. Whether any of these transcripts could function as a primer in DNA replication will be analyzed.

Publications: 85/09 to 86/08

WU, M., KONG, X.F. and KUNG, S.D. 1986. Prokaryotic promoters in the chloroplast DNA replication origin of Chlamydomonas reinhardii. Current Genetics 10 819-822.

WU, M., LOU, J.K., CHANG, D.Y., CHANG, C.H., and NIE, Z.Q. 1986. Structure and function of a chloroplast DNA replication origin of Chlamydomonas reinhardii Proc. Natl. Acad. Sci. USA 83, 6761-6765.

LOU, J.K., CHANG, C.H. and WU, M. Comparative analysis of two chloroplast DNA replication origins of Chlamydomonas (manuscript in preparation).

67.058 CRISO096195 GENES FOR PHOTOSYNTHESIS IN CORN

BOGORAD L; Cellular & Develop Biology; Harvard University, Cambridge, MASSACHUSETTS 02138.

Proj. No.: MAS-8500404 Project Type: CRGO Agency ID: CRGO Period: 01 SEP 85 to 31 AUG 88

Objectives: PROJ 8500404. The overall long range objective of this research program is to identify and understand the mechanisms involved in the control of differentiation of chloroplasts in relation to the maintenance and functioning of the photosynthetic apparatus. The specific objective of this project is to study the proteins at reaction center I of photosynthesis and the plastid genes that code for them.

Approach: We have found and sequenced two large chloroplast genes that code for polypeptides that are about 50% homologous one of which has been shown to be a component of the reaction center of photosystem I. Immunochemical and protein analytical methods are to be used to determine whether the product of the second gene is also a component of the reaction center and if so, what is the relative abundance of the two gene products. Immunochemical methods are also to be used to determine the orientation of the proteins in the chloroplast

membrane and their interactions with other components of the photosynthetic machinery.

67.059 CF TISSUE CULTURE GENETIC SYSTEMS

CRISO136060

PHILLIPS R L; RINES H W; Agronomy & Plant Genetics; University of Minnesota, St Paul, MINNESOTA 55108.

Proj. No.: MIN-13-029 Project Type: CRG0 Agency ID: CRG0 Period: 01 SEP 88 to 31 AUG 91

Objectives: PROJ. 8800422. The focus is on maize tissue cultures, regenerants, and their progeny regarding: a) the role of heterochromatic knobs in chromosome breakage, b) the tissue culture-activation of transposable elements, c) DNA methylation as a cause of variation, and d) relation of culture-induced changes in maturity to heterochromatin and DNA methylation.

Approach: Experiments are proposed that directly test the causative relationship of heterochromatic knobs and chromosome breakage by using strains in culture with various knobs in heterozygous conditions and appropriately marked by RFLP (restriction fragement length polymorphism) markers. Previously generated chromosomal translocations will be cytogenetically analyzed to determine whether they constitute specialized types as predicted from previous results. Several experiments are proposed that test for tissue culture-induced methylation alterations as measured with total DNA from cultures, regenerated plants and their progenies as well as by modification of cryptic AC sequences. The unusual degree of variation in the time of flowering (maturity) among maize regenerated materials will be further documented, and lines previously shown to be variant in maturity will be evaluated for knob constitution and DNA modification.

67.060 TUBULIN GENES OF PLANTS

CRISO097298

SILFLOW C D; SNUSTAD D P; Genetics & Cell Biology; University of Minnesota, St Paul, MINNESOTA 55108.

Proj. No.: MIN-8502429 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 85 to 30 SEP 89

Objectives: Proj 8502429. Our ultimate objective is the structural and functional characterization of the alpha- and beta-tubulin gene families of maize and Arabidopsis. A major emphasis will be to determine whether any of these genes exhibit tissue-specific expression and to identify and characterize the regulatory elements that control their expression.

Approach: Tubulin genes of maize and Arabidopsis will be identified initially by screening cDNA and genomic libraries using heterologous Chlamydomonas tubulin gene probes.

Homologous tubulin gene probes will be used to verify the identification of all members of the tubulin gene families in these two species. Noncoding 3' and 5' regions will be used as gene-specific probes to analyze the patterns of transcription of individual tubulin genes. Tubulin structural gene mutations will be sought using anti-microtubule herbicide-resistant mutants of Arabidopsis.

Progress: 88/01 to 88/12. The maize beta tubulin gene family has 12-15 members, as judged by Southern blot analysis. Only seven different beta-tubulin cDNA clones have been detected, suggesting that some of the genomic beta-tubulin sequences may be pseudogenes. Six-seven beta-tubulin isotypes are also detected in 2-D gels. Two beta-tubulin genes have been sequences and the expression of the genes has been characterized using gene-specific DNA probes hybridized to RNA from various tissues. The beta 1 gene is expressed at high levels in tissue culture cells and in meristematic tissue such as root tips. In contrast, beta 2 gene transcripts are abundant in seedling root but not in root tip. The maize variation tubulins separate into at least 4 different isotypes on 2-D gels. As for the beta-tubulins, the variation-tubulins isotypes show quantitative differences in various tissues. Four different cDNA clones have been isolated and partially characterized. cDNA libraries were constructed from maize pollen and from pollen germinated in vitro for 45 minutes. Pollen is enriched for cytoskeletal proteins; the libraries will be screened for clones corresponding to these proteins. Our characterization of the structure and patterns of expression of the variation-and beta-tubulin gene families of Arabidopsis thaliana has been extended. We now have characterized genomic clones of 5 variation-tubulin genes and 7 beta-tubulin genes.

Publications: 88/01 to 88/12

OPPENHEIMER, D.G., HAAS, N., SILFLOW, C.D. and SNUSTAD, D.P. 1988. The beta-tubulin gene family of Arabidopsis thaliana:preferential accumulation of the beta 1 transcript in roots. Gene 63:87-102.

SILFLOW, C.D., OPPENHEIMER, D.G., KOPCZAK, S.D., PLOENSE, S.E., LUDWIG, S.R., HAAS, N. and SNUSTAD, D.P. 1987. Plant tubulin genes: structure and differential expression during development.

Developmental Genetics 8:435-460.

LUDWIG, S.R., OPPENHEIMER, D.G., SILFLOW, C.D. and SNUSTAD, D.P. 1988. The variation 1-tubulin gene of Arabidopsis thaliana:

LUDWIG, S.R., OPPENHEIMER, D.G., SILFLOW, C.D. and SNUSTAD, D.P. 1988. The variation 1-tubulin gene of Arabidopsis thaliana: primary structure and preferential expression in flowers. Plant Molecular Biology 10:311-321.

JOYCE, C. and SILFLOW, C. 1988. Characterization of tubulin isotypes in zea mays. Molecular Basis of Plant Development UCLA Symposium, Steamboat Springs, CO, March, 1988.

HUSSEY, P.J., LARKIN, J., HUNSPERGER, J. and SILFLOW, C. Expression of two beta-tubulin genes in maize development. In preparation.

67.061 CRISO033929
REVERSE TRANSCRIPTASE AND REPLICATION OF
CAULIFLOWER MOSAIC VIRUS

GUILFOYLE T J; Office of Research Admin.; University of Minnesota, St Paul, ${\bf MINNESOTA}$ 55108.

Proj. No.: MIN-8200386 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 82 to 31 AUG 86

Objectives: Proj. No. 8200386. The primary objectives of this research are to further develop cauliflower mosaic virus (CaMV) DNA as a model system for the study of gene expression, chromatin structure, and replication in higher plants.

Approach: The experimental plan includes: an analysis of the protein components associated with the CaMV minichromosome and the arrangement of these proteins on the CaMV DNA; separation of transcriptionally active and inactive minichromosomes; the isolation of replicative forms of CaMV DNA; and the characterization of the host machinery involved in CaMV DNA replication.

Progress: 84/09 to 85/12. Cauliflower mosaic virus (CaMV) is a double stranded DNA virus of about 8 kilobase pairs. Although this virus offers a potential vehicle for introducing DNA into plant cells, its putative method of replication presents certain obstacles in stabilizing the foreign DNA introduced with this viral vector. We have been studying the mechanisms of transcription and replication of this virus. The current model for this virus is based on reverse transcription of the viral genome where a greater than genome length RNA transcript serves as a template for replication. We have recently found that this replication process may occur in a manner quite similar to replication of Hepatitis B viruses of animals. We have identified putative replication intermediates associated with virions or virus-like particles in infected turnip leaf tissues. These replication intermediates consist of minus-strand DNA(single-stranded DNA complementary to the RNA template used for reverse transcription) extending from "strong-stop" to full genone length. In addition, we have found partially double-stranded DNAs in these putative replication complexes consisting of plus-strand DNA hybridized to minus-strand DNA of partially double-stranded character. These replication complexes also contain an enzymatic activity capable of incorporating deoxyribonucleotide triphosphates into DNA.

Publications: 84/09 to 85/12

MARSH, L., KUZJ, A. and GUILFOYLE, T.J. 1985.

Identification and characterization of cauliflower mosaic virus replication complexes. Analogy to hepatitis B viruses.

Virology 143:221-233.

GUILFOYLE, T.J. 1985. Propagation of DNA

viruses. Methods Enzymol.: In Press.
GUILFOYLE, T.J. 1986. Retro-like viruses in
plants. In: Plant Microbe Interactions (T.
Kosuge and E. Nester, eds.): In Press.

67.062 CRISO134576 OXYGEN RADICALS, OXYINTERMEDIATES AND PROTECTIVE SYSTEMS IN PLANTS

SALIN M L; Biochemistry; Mississippi State University, Mississippi State, MISSISSIPPI 39762

Proj. No.: MIS-6703 Project Type: HATCH Agency ID: CSRS Period: 01 JUL 88 to 30 JUN 93

Objectives: The aim of this project is to learn more about the iron- and manganese-containing superoxide dismutases in plants. By studying the structure and regulation of these potective proteins, an understanding will be gained of how plants cope with oxidative stress in the environment.

Approach: One approach is to determine the amino acid sequence of the iron- and manganese-containing superoxide dismutase in plants. In addition, we will isolate, clone and determine the nucleotide sequence of the gene coding for the superoxide dismutases in plants. We will also initiate studies to determine the control sites of this proteins' synthesis and how alterations in environment might affect gene expression.

Progress: 88/01 to 88/12. Research efforts have focused on determination of the nucleotide sequence of the manganese containing superoxide dismutase from the halophilic bacterium, Halobacterium halobium. A cosmid library of halobial DNA was probed with radiolabeled oligonucleotides constructed to correspond to a sequence of amino acids situated near the N-terminus of the manganese superoxide dismutase. Cosmid DNA was purified from one of the clones that showed hybridization at the highest stringency. A 1.8-kb Pstl fragment of this DNA was subcloned into bacteriophage M13 and tranfected into Escherichia coli JM101. The entire insert containing a 600 base-pair sequence coding for the manganese containing superoxide dismutase and its 5' and 3' flanking regions was sequenced. The derived amino acid sequence of the structural gene showed similarities to other manganese and iron containing superoxide dismutases in normally conserved regions. The 5' region of the sequence showed a number of interesting regions with a similarity to prokaryotic, eukaryotic or uniquely archaebacterial sites. Experiments were also performed to isolate and clone the gene for the iron containing superoxide dismutase from mustard, Brassica campestris. Using a copy of the E. coli iron containing superoxide dismutase gene, we constructed a B. campestris "mini" genomic library.

Publications: 88/01 to 88/12

SALIN, M.L. 1988. Toxic oxygen species and protective systems of the chloroplast.

Physiol. Plant. 72:681-689. SALIN, M.L. and OESTERHELT, D. 1988.

Purification of a manganese-containing superoxide dismutase from Halobacterium halobium. Arch. Biochem. Biophys. 260:806-810.

SALIN, M.L. 1988. Plant superoxide dismutases: a means of coping with oxygen radicals. In: Current Topics in Plant Biochemistry and Physiology. 1988. Vol 7.

(D.D. Randall, D.G. Blevins, W.H. Campbell, eds.) Univ of Mo-Columbia Press.

SALIN, M.L., DUKE, M.V., OESTERHELT, D. and MA, D.P. 1988. Cloning and determination of the nucleotide sequence of the Mn-containing superoxide dismutase gene from Halobacterium halobium. Gene 70:153-159.

BECANA, M. and SALIN, M.L. 1989. Superoxide dismutases in nodules of leguminous plants. Can. J. Bot. "in press".

67.063* CRISCOS1616 GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES

POLACCO J C; Biochemistry; University of Missouri, Columbia, MISSOURI 65211.

Proj. No.: MO-00019 Project Type: HATCH Agency ID: CSRS Period: 01 JUL 85 to 30 JUN 90

Objectives: Since the soybean urease isozymes (ubiquitous and embryo-specific) are differentially regulated and since the ubiquitous form is likely involved in nitrogen fixation we propose to: isolate the structural gene(s) for the embryo-specific and ubiquitous urease isozymes; identify metabolic signals on urease synthesis, induce and select mutations in structural and regulatory genes for each urease, and use cloned urease genes as probes to study the molecular bases of urease regulation and of mutants with altered urease production.

Approach: A urease genomic clone is being used to recover and identify ubiquitous and embryo-specific genes. These will be used to study transcriptional control in cell culture and in both mutant and normal intact plants. Several urease mutants have been recovered.

Progress: 88/01 to 88/12. A fourth urease locus (eu4) has been identified in soybean. A mutation (eu4-aj3) at this locus eliminates urease activity but not antigen in soybean leaves. Roots and callus culture in the eu4-aj3 mutant contain 100 and 40%, respectively, the urease activity of the Williams 82 progenitor. We are testing the hypothesis that eu4 encodes a ubiquitous urease isozyme which is the exclusive species of leaf. These tests include comparison of urease RFLP segregation and the eu4-aj3 trait, and transient expression assay of urease genes of urease in eu4-aj3 leaf propotoplasts. The urease-negative phenotypes of eu2 and eu3-e1 (lacking urease in all vegetative tissue) were shown to be tissue autonomous in graft experiments. The eul (embryo-specific urease locus), eu2 and eu3 loci showed no cosegregation with RFLP's revealed by urease clone E15, corroborating deduced amino acid sequence data indicting that E15 does not encode the embryo-specific urease.

Publications: 88/01 to 88/12
WINKLER RG, DG BLEVINS, JC POLACCO, DD
RANDALL. (1988). Ureid catabolism in
nitrogen-fixing legumes. TIBS 13:97-100.
HOLLAND MA, JD GRIFFIN, LE MEYER-BOTHLING, JC
POLACCO. (1987). Developmental genetics of
the soybean urease isozymes. Dev. Genetics

8:375-387. HOLLAND MA, JO GRIFFIN, JC POLACCO. (1988). Genetics and molecular biology of two developmentally regulated urease isozymes in soybean. Genome 30: Supp 1, abst. 32.14.37 (16th Intntl Cong Genetics). GRIFFIN JO, JC POLACCO. (1988). Molecular genetic analysis of a soybean urease genomic clone. Agronomy Abstracts: 168 Am Soc of Agronomy, Madison, WI. TORISKY RS. May (1988). Characterization of a urease-like genomic clone from soybean. Masters Thesis - UMC. PARK TK. Aug. (1988). Soybean Embryo Lipoxygenases: Molecular Analysis of Null Mutants and Germination-Specific Species. Masters Thesis - UMC.

67.064 CRISO131796 THE REGULATION OF HSP70 GENES DURING STRESS AND DEVELOPMENT IN PLANTS

WINTER J A; Biochemistry; University of Missouri, Columbia, **MISSOURI** 65211. Proj. No.: MO-00490 Project Type: STATE Agency IO: SAES Period: O1 JUL 87 to 30 JUN 92

Objectives: To characterize the differential regulation of plant hsp70 family members and to use heat shock as a tool to study plant biology.

Approach: We have subcloned and sequenced two petunia hsp70 family members. We are isolating others and ultimately will use gene specific probes to characterize the induction properties of each member. We have chimeric genes in transgenic plants utilizing the hsp70 promoter to regulate other genes of interest.

Progress: 88/01 to 88/12. We have characterized hsc70 expression in tomato and isolated a putative p53 genomic clone from a tomato library. We are continuing descriptive work for hsc70 family members (hsc 1 & 2 are sequenced) and ultimately we intend to use the sequence and expression data to correlate specific members tp specific functions.

Publications: 88/01 to 88/12

OUCK, N., MCCORMICK, S. and WINTER, J.

(1989). Hsp 70 cognate expression in vegetative and reproductive organs of Lycopersicon esculentum. PNAS. In Press. ANDERSON, P. and WINTER, J. Benzyladenine induced changes in the population of translatable RNAs from tomato shoots. Submitted to Planta.

WINTER, J., WRIGHT, R., OUCK, N., GASSER, C., ROCHESTER, O., FRALEY, R. and SHAH, D. (1988). The inhibition of petunia hsp70 mRNA processing during CdCl(subscript 2) stress. MGG 211:315-319.

GASSER, C., WINTER, J., HIRONAKA, K. and SHAH, O. (1988). The structure expression and evolution of the 5-endopyruvylshikimate-3-phosphate synthase genes of petunia and tomato. J. Biol. Chem.

67.065 CRISO134567 TRANSCRIPTIONAL REGULATION OF THE GRP1 GENE OF PETUNIA

CONDIT C M; Plant Science and Biochemistry; University of Nevada, Reno, **NEVADA** 89557. Proj. No.: NEVOO430 Project Type: HATCH Agency IO: CSRS Period: O1 JUL 88 to 30 JUN 91

Objectives: The specific objective of this research is to provide detailed and precise information regarding the cis-acting transcriptional regulatory elements of the glycine-rich protein gene of petunia (GRP1). This work should precisely determine those sequences within the 5' flanking region of the GRP1 gene which control this gene's organ-specific expression. In addition, those sequences which regulate the developmental expression and those which cause the enhancement of expression of this gene after wounding will be determined. These studies will expand our knowledge regarding the control of gene expression in plants and will provide basic information on how to specifically target the expression of chimeric genes in transfected plants.

Approach: We will use modern molecular biology techniques to increase the knowledge available regarding control of plant gene expression.

Progress: 88/08 to 88/12. I arrived at the University of Nevada, Reno on 9/17/88 and have only recently established my laboratory in working order. In spite of my recent arrival to an unfinished lab, we have been able to transform petunia leaf strips with the plant transformation vector, pBI 101.3 and regenerate petunia plantlets resistant to kanamycin. We are now in the process of creating chimero constructs containing 5' flanking deletion mutations of the GRP1 gene placed upstream of the B- glucuronidase gene in pBI 101.3. We will within a few months be able to transform petunia leaf strips with these constructs. Once transformed plants containing these constructs are regenerated, we will be able to begin to determine the sequences which control the expression of the GRP1 gene.

Publications: 88/08 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

67.066 CRISO099459 ISOLATION OF GENES THAT ENCODE DNA BINDING PROTEINS

TYE B K; Biochemistry Molecular & Cell Biology; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-181407 Project Type: HATCH Agency IO: CSRS Period: O1 OCT 86 to 30 SEP 89

Objectives: We are interested in proteins that interact with specific ONA structures of yeast chromosomes, such as the replication origins, centromeres and telomeres. We would like to develop a rapid procedure that would allow us to use the specific ONA substrates directly to clone the genes that encode ONA binding proteins that bind specifically to these ONA

substrates from an expression library.

Approach: Recently the technology has been developed for the identification of genes whose products are known. This technology involves the construction of a library of fusion genes in the lambda gt11 expression vector (1). Antibodies specific to the protein of interest are used to screen the library of fusion proteins overproduced in phage plaques fixed on nitrocellulose filters. It has also been shown by others that proteins separated by electrophoresis in polyacrylamide gel, transferred to nitrocellulose filters, still retain their protein conformation, DNA or RNA binding activities (2). We would like to find a condition in which the DNA binding proteins retain their DNA binding activity in phage plaques from the lambda gt11 recombinant DNA library after being transferred to nitrocellulose filters.

67.067 CRISCO96067 MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS

NASRALLAH J B; Plant Biology; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-185315 Project Type: STATE Agency ID: SAES Period: O1 JUN 85 to 30 SEP 88

Objectives: The objectives of this project are to understand at the molecular level the structure and regulation of the self-incompatibility locus in Brassica. A previously-isolated cDNA clone which apparently encodes the S locus product will serve as a starting point for these experiments. The genomic organization of the homologous DNA sequences isolated from plant stocks carrying different S alleles will be compared to determine how these different alleles are generated. Other experiments will be designed to understand the nature of the controls by which the specific regulation of this gene is effected.

Approach: We plan to isolate and characterize DNA sequences from the S locus region by screening, with the cDNA clone, genomic libraries of Brassica DNA prepared from different S genotypes. The nucleotide sequence of important segments of the S locus region will be determined. The transcriptional pattern of the locus will be characterized from cDNA sequence analysis and by S(1) nuclease mapping.

Progress: 88/01 to 88/12. This project is being terminated. Progress during this period is reported under Project NYC-185316.

Publications: 88/01 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

67.068 CRISCO96163 MOLECULAR ANALYSIS OF THE S LOCUS OF BRASSICA

NASRALLAH J B; Plant Biology; Cornell University, Ithaca, **NEW YORK** 14853. Proj. No.: NYC-185416 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 88 to 30 SEP 91

Objectives: The objectives of this project are to understand at the molecular level the regulation of the self-incompatibility genes of Brassica. Genomic sequences coding for self-incompatibility glycoproteins have been cloned, and their pattern of expression analyzed. These cloned sequences will be used to analyze the nature of the controls by which the specific regulation of this gene is effected.

Approach: We plan to carry out plant transformation experiments with cloned S genes, and with constructs containing promoter deletions of various lengths in order to identify the specific sequences responsible for the tight developmental and cell-type specific expression of this gene.

Progress: 88/01 to 88/12. The analysis of the genome organization, structure and expression of sequences from the S locus of Brassica is being pursued. By differential screening of a stigma cDNA library with 32P-labeled cDNA probes synthesized from leaf and stigma poly A+ RNA, we have isolated cDNA clones with homology to the SLSG-encoding cDNA. Thus, two members of the S multigene family are expressed in stigma tissue. We have designated the SLSG structural gene as SLG and the second expressed gene as SLR1. We have shown by nucleotide sequence analysis that SLR1 shares approximately 70% homology with SLG, and by in situ hybridization that, like SLG it is expressed in a very specific cell-type of the stigma, the papillar cell layer of the stigma surface. SLR1 differs from SLG however in a number of characteristics. In particular, we have shown that the SLR1 gene is neither linked to the S locus nor does its sequence show the extensive variability characteristic of the SLG gene. Based on these two criteria, we have concluded that the SLR1 gene is not a determinant of self-incompatibility phenotype. We have not as yet identified the protein product of this gene nor have we determined its function, but the extreme sequence conservation of this gene in various Brassica strains and species implies that its product must play a very basic function in pollen-papillar cell interactions.

Publications: 88/01 to 88/12

NASRALLAH, J.B., YU, S.M. and NASRALLAH, M.E.

1988. Self-incompatibility genes of

Brassica oleracea: Expression, isolation
and structure. Proc. Natl. Acad. Sci. USA
85: 5551-5555.

67.069 CRISOO96934 MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA

NASRALLAH J B; Plant Biology; Cornell University, Ithaca, **NEW YORK** 14853. Proj. No.: NYC-185316 Project Type: CRGO Agency IO: CRGO Period: 15 SEP 85 to 30 SEP 90

Objectives: Proj 8500141. The objectives of this project are to understand at the molecular level the structure and regulation of the self-incompatibility locus in Brassica. A previously-isolated cDNA clone which apparently encodes the S locus product will serve as a starting point for these experiments. The genomic organization of the homologous ONA sequences isolated from plant stocks carrying different S alleles will be compared to determine how these different alleles are generated. Other experiments will be designed to understand the nature of the controls by which the specific regulation of this gene is effected.

Approach: We plan to isolate and characterize DNA sequences from the S locus region by screening, with the cDNA clone, genomic libraries of Brassica ONA prepared from different S genotypes. The nucleotide sequence of important segments of the S locus region will be determined. The transcriptional pattern of the locus will be characterized from cDNA sequence analysis and by S(1) nuclease mapping.

Progress: 88/01 to 88/12. The analysis of the genome organization, structure and expression of sequences from the S locus of Brassica is being pursued. By differential screening of a stigma cONA library with 32P-labeled cONA probes synthesized from leaf and stigma poly A+ RNA, we have isolated cONA clones with homology to the SLSG-encoding cONA. Thus, two members of the \$ multigene family are expressed in stigma tissue. We have designated the SLSG structural gene as SLG and the second expressed gene as SLR1. We have shown by nucleotide sequence analysis that \$LR1 shares approximately 70% homology with SLG, and by in situ hybridization that, like SLG it is expressed in a very specific cell-type of the stigma, the papillar cell layer of the stigma surface. SLR1 differs from SLG however in a number of characteristics. In particular, we have shown that the SLR1 gene is neither linked to the S locus nor does its sequences show the extensive variability characteristic of the SLG gene. Based on these two criteria, we have concluded that the SLR1 gene is not a determinant of self-incompatibility phenotype. We have not as yet identified the protein product of this gene nor have we determined its function, but the extreme sequence conservation of this gene in various Brassica strains and species implies that its product must play a very basic function in pollen-papillar cell interactions.

Publications: 88/01 to 88/12
NASRALLAH, J.B., YU, S.M. and NASRALLAH, M.E.
1988. Self-incompatibility genes of
Brassica oleracea: Expression, isolation
and structure. Proc. Natl. Acad. Sci. USA
85:5551-5555.

67.070 CRISO135669 BIOSYNTHESIS OF ACYL SUGARS AND INSECT RESISTANCE IN LYCOPERSICON

MUTSCHLER M A; STEFFENS J; Plant Breeding & Biometry; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-149308 Project Type: CRGD Agency IO: CRGO Period: O1 JUL 88 to 30 JUN 90

Objectives: PROJ. 8800683. To determine the chemical structures of the acyl sugars in hybrids of L. pennellii and tomato, and relate the structure differences to differences in biological activity. To determine the biosynthetic control of acyl sugar accumulation in L. pennellii and its hybrids. To determine the number, genomic location and specific function ofgenes involved in the control of acyl sugar biosynthesis. To determine the relationship between the genetic control of acyl sugar biosynthesis and aphid resistance.

Approach: Biochemical and insect bioassay techniques will be used for objectives 1 and 2. RFLP techniques, insect bioassays and biochemical techniques for acyl sugar detection and measurement will be used for objectives 3 and 4.

67.071 CRIS0097609
USE OF MOLECULAR MARKERS IN PLANT BREEDING AND
GENETICS

TANKSLEY S 0; Plant Breeding & Biometry; Cornell University, Ithaca, **NEW YORK** 14853. Proj. No.: NYC-149427 Project Type: HATCH Agency IO: CSRS Period: 17 OEC 85 to 30 SEP 89

Objectives: This project will evaluate the use of molecular markers (isozymes, monoclonal antibodies and restriction fragment length polymorphisms) in various aspects of plant breeding. Solanaceous species including tomato, pepper, petunia and tobacco will serve as model systems. Markers will be evaluated as single gene tags and for quantitative genetic analysis of more complex traits. Markers will also be used to study genome organization and evolution in the same species.

Approach: Maps of molecular markers will be developed in tomato, pepper, petunia and tobacco. Markers known to represent homologous sites in the 4 species will be used for making inferences about how the genomes have been rearranged since species divergence and to measure levels and rates of gene duplications/deletion. Hybrid crosses and segregating generations will be used for mapping as well as studying linkage relationships of quantitative as well as qualitative traits with molecular markers.

Progress: 88/01 to 88/12. Research has concentrated on developmemnt and application of restriction fragment length polymorphisms (RFLPs) in tomato, potato and rice. The maps of these species now contain more than 200 markers each. The RFLP map in tomato has been used to

locate genes for fruit characters inherited from a wild species. Most important economically are the genes for soluble solids. Using RFLP analysis and fie3ld testing, we have located more than 5 putative genes for high soluble solids. Three of those have been tested over 3 growing seasons and still look promising. Currently, near-isogenic lines are being tested that contain different combinations of these genes. In rice we have used the RFLP map to search for resistance to bacterial blight, leaf hopper and rice blast resistance. Near-isogenic lines for each disease resistance gene have been used to screen large numbers of mapped RFLP clones. Putative positive 'hits' have been recorded from approximately half of the targeted genes. The ultimate goal is to use RFLP fingerprinting to aid in the breeding of these important disease resistance genes.

Publications: 88/01 to 88/12

TANKSLEY, S.D. and J. HEWITT. 1988. Use of molecular markers in breeding for soluble solids content in tomato - a re-examination. Theor. Appl. Genet. 757:811-823.

BERNATZKY, R., E. PICHERSKY, V.S. MALIK, and S.D. TANKSLEY. 1988. CR1 - A dispersed repeated element associated with the CAB-1 locus in tomato. Plant Mole. Biol. 10:423-434.

TANKSLEY, S.D., R. BERNATZKY, N.L.LAPITAN, and J.P. PRINCE. 1988. Conservation of gene repertoire but not gene order in pepper and tomato. Proc. Natl. Acad. Sci. 85:6419-6423.

MCCOUCH, S.R., G. KOCHERT, Z. YU, Z. WANG, G.S. KHUSH, W.R. COFFMAN, and S.D. TANKSLEY. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. (in press).

BONIERBALE, M., R.L. PLAISTED, and S.D. TANKSLEY. 1988. RFLP maps of potato and tomato based on a common set of clones reveal modes of chromosomal evolution. Genetics 120:1085-1103.

PATERSON, A.H., E.S. LANDER, J.D. HEWITT, S. PETERSON, S.E. LINCOLN, and S.D. TANKSLEY. 1988. Resolution of quantitative traits into Mendelian factors by using a complete RFLP linkage map. Nature 335:721-726.

67.072 CRISO131944 ANALYSIS OF PROTEINS ASSOCIATED WITH CYTOPLASMIC MALE STERILITY

HANSON M R; Section of Genetics & Develop; Cornell University, Ithaca, **NEW YORK** 14853. Proj. No.: NYC-186302 Project Type: CRGO Agency ID: CRGO Period: O1 SEP 87 to 31 AUG 89

Objectives: PROJECT 8700572. To determine the molecular mechanism of cytoplasmic male sterility, a defect in pollen development encoded by the mitochondrial genome. Discover how the gene product of a mitochondrial gene affects developing microspores.

Approach: Experiments to be performed: Obtain N-terminal sequence data for CMS-associated polypeptide isolated from Petunia; Make

antibodies to CMS-associated polypeptide; Compare amino acid and DNA sequence data for CMS-associated gene product and gene; Identify the location of CMS-associated polypeptide and related proteins by fractionation and microscopic studies in conjunction with immunological methods.

Progress: 88/01 to 88/12. Further immunological studies were carried out to characterize the products of the Petunia gene, termed S-pcf, which is associated with cytoplasmic male sterility (CMS). Synthetic peptide antibodies which recognize two polypeptides (20kD and 25kD) in CMS lines' mitochondria recognized only the 20kD polypeptide in fertile lines' mitochondria. A portion of the pcf gene coding region was expressed in E. coli, and additional polyclonal antibodies were obtained which recognized the 20 and 25kD polypeptides. In fertility-restored lines carrying the CMS cytoplasm but a single dominant nuclear restorer allele, the 20kD polypeptide was observed, but the 25kD polypeptide was much reduced. These experiments indicate that the 25kD polypeptide is a pcf gene product whose expression is affected by the presence of a nuclear restorer allele. Thus the pcf locus has been found to be associated with CMS in two ways: it segregates with the sterility phenotype in somatic hybrids containing recombinant mtDNAs, and its expression is modified by the same nuclear gene which confers a fertile phenotype on lines carrying the CMS-encoding cytoplasm.

Publications: 88/01 to 88/12

HANSON, M.R., YOUNG, E.G. and ROTHENBERG, M.
1988. Seq. & expr. of a fused mitoch. gene, assoc'd w/ Petunia cytoplas. male sterility, compared with normal mitochondrial genes in fertile and sterile plants. PhilTrR.Soc.319:199-208.

67.073 CRISO09930 THE ROLE OF POLYAMINES DURING STRESS-INDUCED ALTERATIONS IN GENE EXPRESSION

HIATT A C; Cold Spring Harbor Laboratory; P 0 Box 100, Cold Spring Harbor, **NEW YORK** 11724. Proj. No.: NYR-8600064 Project Type: CRG0 Agency ID: CRG0 Period: O1 AUG 86 to 31 JUL 89

Objectives: PROJECT 8600064. Careful regulation of polyamine metabolism in plants may be an important aspect of floral development as well as an important response to stressful growth conditions. The overall objective of this research is to define the relationship between polyamine metabolism and stress-induced alterations in gene expression; we need to understand how regulation of the polyamine pathway contributes to the growth characteristics and development of plants in various environments.

Approach: Regeneration into whole plants of tobacco cell culture mutants with characterized defects in polyamine metabolism and reintroduction of genes responsible for polyamine synthesis into wild type and mutant plants will be the primary approaches to

understanding the importance of polyamine metabolism during development.

Progress: 88/01 to 88/12. Sequencing and regulation of ornithine carbamyltransferase from higher plants. Since OTC is a key enzyme of the urea cycle as well as being a potential regulator of the polyamine pathway, we have isolated a full length cDNA from Arabidopsis thaliana and have nearly completed the sequencing Isoltion of putative ARGdc cDNAs from Arabidopsis. Antibodies to E. coli ARGdc may crossreact with plant ARGdc. We have used the serum to isolate a number of cDNAs from our Arabidopsis GT-11 library and our currently analyzing them (cross homology, expression in bacteria) to determine if they contain ARGdc sequences. Metabolic biology of putrescine production in maize calli. Using a variety of genetically defined calli we have demonstrated that there are two distinct populations of intracellular putrescine. The first, derived from ornithine, is composed exclusively of free polyamine the second, derived from arginine, gives rise to free putrescine as well as to all of the conjugated putrescine attached to the hydroxycinnamic acids. Heterologous and antisense gene expression in tobacco. Our first transformants are aimed at expressing the mouse ORNdc cDNA in tobacco to see if the mouse protein is stable in the tobacco cell and if it contributes to the overall ornithine decarboxylase activity. We have also expressed a cDNA (pAD5) which is stress-regulated as well as being regulated by polyamines in the same manner as ARGdc.

Publications: 88/01 to 88/12
HIATT, A. and MALMBERG, R.L. 1988.
Utilization of putrescine in Tobacco Cell
Lines Resistant to Inhibitors of Polyamine
Synthesis. Plant Physiol. 86, 441-446.
HIATT, A. 1988. Polyamine Synthesis in Maize
Cell Lines. Plant Physiol., submitted.

67.074 CRISO034117 CHARACTERIZATION OF PLANT GENES ENCODING PHOTOSYNTHETIC MEMBRANE PROTEINS

CASHMORE A R; Department of Cell Biology; Rockefeller University, New York, **NEW YORK** 10021.

Proj. No.: 8300763 Project Type: CRG0 Agency ID: CRG0 Period: 01 AUG 83 to 31 JUL 87

Objectives: Proj 8300763. To isolate and characterize the nuclear genes from pea encoding the constituent polypeptides of the light-harvesting chlorophyll a/b protein complex.

Approach: Cloned genes will be isolated and sequenced. By hybrid-selection studies the genes will be related to the different chlorophyll a/b binding polypeptides. Hybridization studies will be carried out to define the expression of the individual chlorophyll a/b binding protein genes.

67.075 CRISO130049 PLANT GENE REGULATION DURING NITROGEN ASSIMILATION

CORUZZI G M; Lab of Plant Molecular Biology; Rockefeller University, New York, **NEW YORK**

Proj. No.: NYR-8601670 Project Type: CRG0 Agency ID: CRG0 Period: 15 SEP 86 to 30 SEP 87

Objectives: PROJECT 8601670. To examine the molecular events in the biosynthesis of chloroplastic glutamine synthetase (GS) in leaves of higher plants. We will determine: the genome of origin (nuclear vs. chloroplast); the primary structure of the GS mRNA and polypeptide; the steps in the synthesis of the polypeptide and its import into chloroplasts.

Approach: The approach we will use is to: isolate full length cDNA clones encoding chloroplastic GS from a leaf cDNA library; determine the DNA sequence; define a chloroplast "transit peptide" by in vitro synthesis and uptake of the encoded GS polypeptide into chloroplasts; use the cDNAs as molecular hybridization probes to characterize the GS transcripts in various organs as well as the gene(s) in the nuclear genome.

Progress: 86/09 to 87/09. A full length cDNA clone for chloroplast glutamine synthetase (GS2) has been isolated and characterized by nucleotide sequence analysis. The deduced amino acid sequence encodes a GS2 polypeptide which is 75% homologous to that deduced for cytosolic GS, and contains an amino terminal chloroplast transit peptide. In vitro chloroplast uptake experiments have shown that chloroplast GS2 is synthesized as a precursor polypeptide (49 kd), which is imported into chloroplasts and processed to the mature size of chloroplast stromal GS2 (44 kd). Light has been shown to induce the accumulation of GS2 polypeptide and mRNA, in a phytochrome mediated response. These results have shown that is pea, the chloroplast form of glutamine synthetase (GS2), is encoded by a nuclear gene which is highly homologous to those which encode the cytosolic forms of GS (GS1, GSn). The GS gene family is unique from other plant multigene families studied to date. For GS, individual genes encode distinct gene products which are targeted to different subcellular compartments (chloroplast or cytosol). In addition, the individual GS genes are each regulated by distinct factors in vivo. The nucleotide sequence data suggests that chloroplastic and cytosolic GS genes are derived from a common ancestor, and provide molecular data concerning the evolution of the plant cell.

Publications: 86/09 to 87/09

TINGEY, S.V., TSAI, F.Y., EDWARDS, J.W.,
WALKER, E.L. and CORUZZI, G.M. (1988)
Chloroplast and Cytosolic Glutamine
Synthetase are Encoded by Homologous
Nuclear Genes which are Differentially
Expressed in Vivo. (Submitted to J. Biol.
Chem.

67.076 CRISO136740 RESEARCH ON THE INTRASPECIFIC VARIATION IN CENOCOCCUM GEOPHILUM FR.

WANG C J K; Environ & Forest Biology; State University of New York, Syracuse, **NEW YORK** 13210.

Proj. No.: NYZ-2112-01-016 Project Type: STATE Agency ID: OCI Period: 12 APR 88 to 11 APR 89

Objectives: To characterize and differentiate isolates of the mycorrhizal fungus Cenococcum geophilum fr. obtained worldwide.

Approach: Comparison of restriction fragment length polymorphisms in ribosomal DNA, vegetative compatibility groups, and cultural and physiological characters.

67.077 CRISO099298 MAPPING THE SYMBIOSIS GENES OF PEA

LARUE T A; WEEDEN N; Boyce Thompson Institute; Tower Road, Ithaca, **NEW YORK** 14853.

Proj. No.: NYR-8601280 Project Type: CRG0 Agency ID: CRG0 Period: O1 AUG 86 to 31 JUL 89

Objectives: PROJECT 8601280. We induced mutants of pea (Pisum sativum) which are non-nodulating or form few nodules. Some have now been characterized as single gene, nonallelic mutations at symbiosis (sym) loci. In this study we will map these sym genes in the pea chromosome by establishing linkage to known isozyme loci.

Approach: Horizontal starch gel electrophoresis will be used in combination with a set of 25 isozyme assays to map the sym loci on the pea genome. Tester lines have been developed which consist of different sets of isozyme variauts, allowing most of the genome to be covered in two crosses. Thus, each mutant will be reciprocally crossed with two tester lines, and the hybrids allowed to self pollinate to produce F(2) generations. These F(2)generations will be scored for segregating morphological and isozyme polymorphism as well as for the ability to nodulate. Once a sym mutant has been located to a specific chromosome, a third cross will be made with the tester line for that chromosome.

Progress: 87/08 to 88/07. Mapping the symbiosis (sym) genes of Pisum sativum is on schedule. In this second year, four were mapped and another linked to an as-yet unmapped gene. Work began on developing a RLFP map of the normal parent cultivar 'Sparkle'.

Publications: 87/08 to 88/07 NO PUBLICATIONS REPORTED THIS PERIOD.

67.078 CRISO132959 GENE REGULATION IN MAIZE ENDOSPERM

BOSTON R S; Botany; North Carolina State
University, Raleigh, NORTH CAROLINA 27695.
Proj. No.: NCO5648 Project Type: STATE
Agency ID: SAES Period: O1 AUG 87 to 30 SEP 88

Objectives: To isolate genes encoding B32 and B70, two endosperm proteins affected by mutations that also alter synthesis and accumulation of zeins, the major storage proteins of maize. To compare expression of these genes in normal corn and in corn containing endosperm mutations. To determine the function of B32 and B70 proteins.

Approach: Genes will be isolated using standard recombinant DNA techniques. Maize kernels will be harvested at different develoomental stages, quick-frozen and stored until nucleic acids can be extracted for analysis of gene expression by S1-mapping, nuclear run-off transcription and Northern blotting. The function of B32 and B70 will be investigated by determining their subcellular localization and by analyzing the effect of introducing the genes into protoplasts.

Progress: 87/08 to 88/09. Studies to isolate genes encoding two developmentally regulated proteins in maize endosperm have been initiated. These proteins, b-32 and b-70 are synthesized in endosperm tissue of maize during kernel development, cDNA clones were isolated from lambda expression libraries by screening with b-32 and b-70 antibodies. The b-32 cDNA clone is similar but not identical to another b-32 clone recently reported. We have used this clone to analyze accumulation of b-32 RNA in developing endosperm. No b-32 RNA is detectable at early (less than 8 days after pollination, DAP) stages of kernel development, levels increase between 10 and 24 DAP and decline during kernel maturation and desiccation. In the endosperm mutant, opaque-2, appearance of b-32 mRNA is delayed and reduced compared to levels in normal tissue. We are now using the b-32 cDNA clone to probe a maize library for the corresponding genomic clones. Characterization of the b-70 cDNA clones are still in the initial stages. We have begun preliminary analysis of protein body structure during kernel development by analyzing developing kernels with scanning electron microscopy. There are marked differences in structure in protein bodies from normal maize and from the floury-2 mutant of maize which overexpresses the b-70 protein.

Publications: 87/08 to 88/09 NO PUBLICATIONS REPORTED THIS PERIOD.

67.079 CRISO135053 REGULATION OF GENE EXPRESSION IN DEVELOPING MAIZE KERNELS

BOSTON R S; Botany; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO6065 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 91

Objectives: To isolate genes encoding B32 and B70, two endosperm proteins affected by mutations that also alter synthesis and accumulation of zeins, the major storage proteins of maize. To compare expression of these genes in normal corn and in corn containing endosperm mutations. To determine the function of B32 and B70 proteins.

Approach: Genes will be isolated using standard recombinant DNA techniques. Maize kernels will be harvested at different develomental stages, quick-frozen and stored until nucleic acids can be extracted for analysis of gene expression by S1-mapping, nuclear run-off transcription and Northern blotting. The function of B32 and B70 will be investigated by determining their subcellular localization and by analyzing the effect of introducing the genes into protoplasts.

67.080 CRISO096202 ISOLATION AND CHARACTERIZATION OF GENES REGULATED BY LIGHT

THOMPSON W F; Botany; North Carolina State University, Raleigh, NORTH CAROLINA 27695. Proj. No.: NC-8500128 Project Type: CRGO Agency ID: CRGO Period: 15 SEP 85 to 15 SEP 86

Objectives: PROJ 8500128. We have been studying a group of about 20 genes represented by cDNA clones derived from mRNAs which change their abundance in pea (Pisum Sativum L.) buds exposed to red light treatments. Most of the light responses are mediated by the phytochrome system although some genes also exhibit blue light responses. We propose to obtain genomic clones for a selected subset of these genes and to use these clones to extend our knowledge of the molecular biology of the light responses.

Approach: We will carry out an extensive cloning/sequencing program in which we will obtain and analyze genomic clones of up to eight different genes. Emphasis will be placed on several genes which appear to be represented by only 1-2 copies in the pea genome, since a low copy number will greatly facilitate experimental design and interpretation. We also propose with respect to examine possible changes in the intracellular localization of various transcripts during the light response. These experiments will be initiated with our existing cDNA clones as probes and developed further using genomic clones as they become available.

67.081 CRISO097731 PHOTOREGULATED GENE EXPRESSION IN CHLOROPLASTS

THOMPSON W F; Botany; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NC-8502364 Project Type: CRGO Agency ID: CRGO Period: 30 SEP 85 to 01 OCT 87

Objectives: Proj 8502364. Photoregulation of Gene Expression in Chloroplasts.

Approach: We propose to extend our recent investigations of light controlled expression of nuclear genes to include chloroplast genes as well. We will prepare a transcript map of the pea chloroplast genome for dark- and light-grown plants, using cloned probes averaging 2-4 Kb in length and hybridizing them to Northern blots. From this analysis we will select regions of the genome and specific genes to examine in more detail. In this phase of the work we propose to investigate the effects of wavelength, intensity, and duration of incident light in some detail. It is hoped that this work will provide a basis for future studies in which the biochemistry behind the light effects can be studied using in vitro systems.

67.082 CRISO131015 PHOTOREGULATED GENE EXPRESSION IN CHLOROPLASTS

THOMPSON W F; Botany; North Carolina State University, Raleigh, NORTH CAROLINA 27695.

Proj. No.: NCO9345 Project Type: CRGO Agency ID: CRGO Period: 15 DEC 86 to 31 DEC 89

Objectives: PROJECT 8603248. To prepare a map of transcripts from both known and unknown genes in the pea chloroplast genome and to characterize changes in transcript patterns which may occur during chloroplast development.

Approach: The basic transcript localization work will involve "Northern" hybridization analysis, using a total of about 100 chloroplast DNA clones, including more than 20 representing identified genes. The same clone bank will be used in further attempts to map transcript initiation sites using chloroplast RNA capped in vitro and techniques such as hybrid selection and S-1 nuclease analysis.

Progress: 88/01 to 88/12. We have published the transcript mapping and developmental work described in last year's report (Woodbury et al., 1988) and are now in the process of mapping as many actual transcription initiation sites as possible. We are exploiting the fact that plastid mRNAs are not capped in vivo as cytoplasmic mRNAs are, so we can introduce label specifically at the initiation site by carrying out in vitro capping reactions with labeled GTP. This reaction labels only those 5' ends which still contain a triphosphate, and thus will not label ends resulting from cleavage events in processing or during isolation. We think we can identify the approximate location of about 30 initiation sites in this way and may be able to locate some of these more precisely. Initiation sites have previously been mapped for 3 chloroplast genes.

Publications: 88/01 to 88/12
PALMER, J. D., B. OSORIO, K. J. ALDRICH, and
W. F. THOMPSON. 1987. Chloroplast DNA
evolution among legumes: Loss of a large
inverted repeat occurred prior to other

sequence rearrangements. Curr. Gen. 11, 275-286.

PALMER, J.O., B. O. OSORIO, and W. F.
THOMPSON. 1988. Inversions in legume
chloroplast DNAs. Curr. Gen. 14, 65-74.
WOODBURY, N. W., L. L. ROBERTS, J. D. PALMER,
and W. F. THOMPSON. 1988. A transcription
map of the pea chloroplast genome. Curr.
Gen. 14, 75-89.

67.083 CRISO132702 ISOLATION AND CHARACTERIZATION OF GENES REGULATED BY LIGHT

THOMPSON W F; Botany; North Carolina State
University, Raleigh, NORTH CAROLINA 27695.
Proj. No.: NCO6030 Project Type: HATCH
Agency IO: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: To obtain and characterize genomic clones for genes encoding mRNAs which change their abundance in pea buds exposed to various light treatments.

Approach: Cloning/sequencing of several different genes. Will also examine possible changes in the intracellular localization of various transcripts.

Progress: 87/10 to 88/12. We now have evidence that levels of mRNA encoding ferrodoxin I are regulated in a manner quite different from the transcriptional control mechanisms operating in the only other well studied plant gene systems. Using transgenic plants in which various parts of the Fed 1 gene have been combined with complementary portions of a normally nonresponsive gene we can show that elements required for normal light effects on the level of this mRNA are within the Fed-1 gene itself rather than in 'upstream' regions where transcriptional regulatory elements are normally located. Only two interpretations seems likely. One is that Fed-1 contains its transcriptional regulatory elements within itself. The other, which we presently favor, is that light regulates Fed-1 mRNA levels by controlling mRNA degradation rather than transcription. In other work, we have prepared gene specific oligonucleotides to use as probes in studies on the expression of individual members of the Cab mulitgene family. We have also identified a cONA encoding a 21 Kd polypeptide from photosystem I, and characterized another which encodes a protein similar to several lectins. The latter mRNA is remarkable in being present only in actively growing regions of the pea shoot. Thus the protein it encodes might play a role in cell division or wall synthesis in rapidly growing plant tissue. We have also nearly finished developing techniques for isolation of nuclei suitable for differential fluorescent staining and FACS analysis of nucleolar activity.

Publications: 87/10 to 88/12

HOWORWITZ, B. A., W. F. THOMPSON, and W. R. BRIGGS. 1988. Phytochrome regulation of greening in Pisum: chlorophyll accumulation and abundance of mRNA for the light-harvesting chlorophyll a/b binding proteins. Plant Phys. 86, 299-305.

PALMER, J. O., B. O. OSORIO, and W. F. THOMPSON. 1988. Inversions in legume chloroplats ONAs. Curr. Gen. 14, 65-74. SAGAR, A. O., B. A. HOROWITZ, R. C. ELLIOTT, W. F. THOMPSON, and W. R. BRIGGS, 1988. Light effects on several chloroplast components in Norflurazon-treated pea seedlings. Plant Phys. 88, 340-347. THOMPSON, W. F., R. B. FLAVELL, J. C. WATSON, and L. S. KAUFMAN. 1988. Chromatin structure and expression of plant ribosomal RNA genes. In: G. Kahl, ed., Architecture of Eukaryotic Genes, VCH Verlagsgesellschaft, Weinheim, pp 385-396. THOMPSON, W.F. et al. 1988. Patterns of phytochrome induced gene expression in etiolated pea buds. In: G. L. Steffans and T. S. Rumsey, eds., Biomechanisms Regulating Growth and Oevelopment, pp 269-284. WOOOBURY, N. W., L. L. ROBERTS, J. O. PALMER, and W. F. THOMPSON. 1988. A transcription map of the pea chloroplast genome. Curr. Gen. 14, 75-89.

67.084 CRISO093119 STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED DURING NITROGEN FIXATION

CURTIS S E; Genetics; North Carolina State
University, Raleigh, NORTH CAROLINA 27695.
Proj. No.: NCO3866 Project Type: HATCH
Agency IO: CSRS Period: O1 JUL 84 to 30 SEP 89

Objectives: To study the regulation of photosynthetic genes during nitrogen fixation. In particular, cyanobacterial genes whose products participate in the Calvin cycle, photosystem II and ATPase complex will be studied with regard to structure, expression and evolution.

Approach: Photosynthetic genes will be isolated using heterologous probes from plants and bacteria. The structure of these genes will be studied by determining their sequence and relative organization. Gene regulation will be studied by Northern blot analysis and transcription initiation mapping.

Progress: 88/01 to 88/12. transcription of the genes encoding the subunits of ovine follicle-stimulating hormone. Mol. Endocrinol. 2:641-649. In past work in our laboratory, seventeen genes that encode polypeptides involved in photosynthesis have been isolated and their sequences determined. We have designed experiments which will allow us to define promoter sequences of these genes using functional assays. The system currently in use employs the E. coli chloramphenicol acetyl transferase (CAT) gene as a reporter. ONA fragments of varying lengths upstream from each of the operons have been placed in front of the CAT gene. Expression of CAT requires the presence of an Anabaena promoter in the sequences cloned 5' to it. These constructions were placed into a shuttle vector which can be propagated in either Anabaena or E. coli. The shuttle vector constructs were mobilized into Anabaena via conjugation with E. coli.

Exconjugant cells carrying constructs with sequences from each operon are currently being assayed for CAT activity. Initial results indicate that the system is working, i.e. constructs carrying fragments in the proper orientation give significantly higher levels of CAT activity than constructs containing either no insert or the fragment in the wrong orientation.

Publications: 88/01 to 88/12
CURTIS, S.E. 1988. Structure, Drganization and Expression of Cyanobacterial ATP
Synthase Genes. Photosyn. Res. 18:223-244.
McCARN, D.F., WHITAKER, R.A., ALAM, J., VRBA, J.M. and CURTIS, S.E. 1988. The Genes
Encoding the Alpha, Gamma, Delta and Four FO etc. J. Bacteriol. 170:3448-3458.

67.085* CRISOO91503
RECOMBINANT DNA APPROACHES TO PLANT GENE
STRUCTURE AND GENOME DIVERSITY

DLESDN A E; BERRYHILL D L; KDFDID K D; Biochemistry; North Dakota State University, Fargo, **NORTH DAKOTA** 58105.

Proj. No.: NDO1228 Project Type: HATCH Agency ID: CSRS Period: 01 NDV 83 to 30 SEP 88

Objectives: Clone stress response genes from selected plant species and determine the nucleotide sequences of their polypeptide reading frames and flanking regulatory regions. Elucidate the dynamics of expression of stress response genes under various stress conditions by use of recombinant DNA probes. Clone and determine the sequence of nuclear genes for ribosomal RNA from higher plants. Assess mitochondrial diversity in wheat with recombinant DNA techniques. Determine the effects on wheat mitochondrial DNA of growth in tissue culture and regeneration.

Approach: A generalized stress-response plant enzyme (RNase I) will be purified, partially sequenced (Edman method), and a synthetic oligonucleotide probe prepared. Clones from cDNA and genomic libraries will be isolated, sequenced, and used as probes of specific mRNA levels in stressed plants. A previously cloned maize rRNA gene in a lambda vector will be subcloned into plasmid and M13 phage vectors, and sequenced by the dideoxy method. Mitochondrial DNA will be isolated from several species of Triticum and Aegilops and restriction maps prepared. Sequence homologies will be determined by blotting procedures. Restriction maps of mitochondrial DNA from a single species subjected to tissue culture and regeneration will also be compared.

Progress: 83/11 to 88/09. Mitochondrial DNA has been isolated from five wheat cultivars. Each has an AABBDD nucleus, but the cytoplasmic genomes came from Aegilops squarrosa, Haynaldia villosa, T. aestivum, T. timopheevi, and T. turgidum. The DNA preparations were examined with restriction endonucleases BamHI, EcoRI, HindIII, and XhoI. Labeled probes used for this analysis were from T. aestivum or sorghum mitochondrial DNA. The results indicated that Haynaldia villosa was the B genome donor to T.

turgidum and T. aestivum. In other work, a lambda clone of the nuclear ribosomal gene region of maize has been subcloned into Escherichia coli plasmid vectors. Shotgun fragments were cloned into a phage vector, and these were sequenced by the dideoxy method. This gene family is present as tandem repeats on the chromosome. The transcript encodes, in a 5' to 3' direction, 17S, 5.8S, and 26S ribosomal RNAs, with internal transcribed spacers 1 and 2 flanking the 5.85 region. Clones containing the 5.85 and spacer regions were used as probes for RFLP analysis of corn DNA. These studies indicated that little variation exists in the internal spacer regions of the multiple copies of this gene family. Sequencing of the 26S region was completed. Comparison of the alpha-sarcin domain of this RNA indicated that the 14-base core was the same as that of all other eukaryotes tested. The base flanking the 5'-end of the core is C in the case of all animals tested, whereas U is present at this position in corn, yeast and

Publications: 83/11 to 88/09

MESSING, J.; CARLSDN, J.; HAGEN, G.;

RUBENSTEIN, I.; and DLESDN, A. 1984.

Cloning and sequencing of the ribosomal RNA genes in maize: The 17S region. DNA 3:31-40.

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67.086 CRISO133651 AUXIN METABOLISM IN ARABIDOPSIS: A GENETIC APPROACH

TDWN C D; Western Reserve University, Cleveland, **OHIO** 44106.
Proj. No.: DHDR-8701103 Project Type: CRGD Agency ID: CRGD Period: O1 SEP 87 to 31 AUG 89

Objectives: PRDJ. 8701103. Characterize tryptophan and auxin mutants of Arabidopsis: Characterize and quantitate free and bound auxins and their metabolism in Arabidopsis; Isolate mutants in auxin conjugate metabolism; Clone and characterize mutationally-identified genes.

Approach: Measure growth rates, analog cross-resistance, enzyme actities, tryptophan and IAA content of mutant plants and callus. Map mutant genes. Extract, HPLC purifyand use GC-MS to quantitateauxin and auxin conjugates in wild-type plants. Screen potentially toxic auxin conjugates as selective agents and apply selection procedures to mutagenized seed stock. Attempt rescue of selectable (dominant) genes by direct transformation of tobacco.

Progress: 88/01 to 88/12. We have isolated mutants resistant to alpha-methyl tryptophan (alpha-MT) and begun to characterize one of them. Mutant seedlings are resistant to 25uM alpha-MT, while callus is resistant to at least 100uM alpha MT. Genetic analysis indicates that the initial isolate was homozygous, and that the mutation is dominant. The mutant shows cross-resistance to 5-methyl tryptophan (5-MT) but is as sensitive as wild-type to 5-methyl anthranilate. A genomic library from the mutant DNA has been constructed in an

Agrobacterium-based binary cosmid vector, and is being used to transform tobacco from alpha-MT sensitivity to resistance, thereby selecting for the resistance allele. Using procedures described by Chen et al (Plant Phys. 86, 822, 1988), the amounts of free, ester- and amide-conjugated IAA in 3-4 week old Arabidopsis plants were determined to be 1.0 mu g, 2.3 mu g and 12.2 mu g per gram fresh weight of whole plant (predominantly leaf) tissue. Similar, unusually high values are now being reported for Arabidopsis by others. A mutant isolated as resistant to IAA ethyl ester was found to be resistant to other auxins (NAA, 2,4,-D) also. The mutant is unable to form callus on medium containing auxin at concentrations optimal for growth of wild-type material. Different parts of the mutant plants demonstrated different levels of auxin resistance, with leaves showing greatest, roots intermediate and stems least resistance. The mutant shows normal root gravitropism.

Publications: 88/01 to 88/12
KREPS, J.A. and TDWN, C.D. Mutants of
Arabidopsis thaliana resistant to
alpha-methyl tryptophan. Abstract 267,
13th International Conference on Plant
Growth Substances, 1988.
TDWN, C.D. and PERSINGER, S.M. Tissue
Specific Expression of Auxin Resistance in
a Mutant of Arabidopsis thaliana. Abstract
279, 13th International Congress on Plant
Growth Substances, 1988.

67.087 0025044 BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION

BISHDP N I; Botany & Plant Pathology; Dregon State University, Corvallis, **OREGON** 97331. Proj. No.: DREO0656 Project Type: STATE Agency ID: SAES Period: 01 SEP 86 to 31 AUG 91

Objectives: Two groups of chloroplast proteins, one membrane-bound and the other soluble, are essential for the water splitting reactions of photosynthesis. The principle objectives of the proposed research are to gain further information about the role of the nuclear-coded, soluble peptides in governing water photolysis through their influence on C1 and Ca concentrations in the oxygen-evolving-complex of the chloroplast and to evaluate further the potential role of an intrinsic chloroplast membrane polypeptide in the binding and coordination of the requisite manganese of the oxygen-evolving-complex.

Approach: The objectives of the proposed research will be evaluated through a biochemical-genetic approach utilizing the single celled green algae, Scenedesmus obliquus and Chlamydomonas reinhardtii and biochemical studies on isolated chloroplasts of these algae and mutant phenotypes and of higher plants. Desired mutant phenotypes in which the mechanism of water photolysis has been affected either through alteration of the respective chloroplast or nuclear genome are available for developmental studies. Whole cell and isolated chloroplast reactions involving photosystem II

will be measured to evaluate the state of the oxygen-evolving complex. Synthesis of membrane components will be evaluated by S labeling of chloroplast proteins coupled with electrophoresis and autoradiography.

Progress: 88/01 to 88/12. The chloroplast genome encodes for five major intrinsic, water insoluble proteins that are essential for the function of photosystem II (PS-II). These include two chlorophyll-binding apoproteins (48 and 44 kilodaltons), two reaction center polypeptides (D(1)- and D(2)-proteins of 32-34and 30-32 kDa, respectively) and cytochrome b-559 (10kDa). Assembly of these components during the light-induced development of PS-II apparently requires the coordinated synthesis of each of these components. Current studies on mutant phenotypes of the green alga, Scenedesmus obliquus, which develop to (1) a normal phenotype or (2) to a phenotype lacking the D(1)-polypeptide have demonstrated that the inability to synthesize the D(1)-protein also prevents the formation of the light-harvesting, chlorophyll-protein complexes directly associated with the reaction center components. Although the ability of this phenotype to synthesize the apoprotein (48 kDa) in unimpaired, the addition of pigments (chlorophyll a and carotenes) seems either to not occur or to be subject to a rapid turnover. These alternatives are currently being evaluated with the original strains and several secondary developmental phenotypes.

Publications: 88/01 to 88/12
BISHDP, N.I., HUMBECK, K. and SENGER, H.
1989. Adaptation of the photosynthetic apparatus in a mutant of Scenedesmus obliquus lacking the light harvesting system. J. Plant Physiology. In Press.
HUMBECK, K. and BISHDP, N.I. 1989.
Biosynthesis of photosystem II-polypeptides and development of oxygen evolution in greening mutants of Scenedesmus.
Photochemistry and Photobiology. In Press.

67.088 THE PHYCOCYANIN GENES OF AGMENELLUM OUADRUPLICATUM

STEVENS S E JR; Biochemistry & Microbiology; Pennsylvania State University, University Park, **PENNSYLVANIA** 16802.

Proj. No.: PENR-8100577 Project Type: CRGD Agency ID: CRGD Period: 15 AUG 82 to 30 NDV 85

Objectives: Proj. No. 8200719. We will study the structure and function of the phycocyanin apoprotein DNA and RNA coding sequences from Agmenellum quadruplicatum. Clones of the phycocyanin genes established in Escherichia coli will be used as starting material construction of a restriction map and determination of the nucleotide sequence of these genes will be done. A transcription map of the phycocyanin genes will be constructed. Directed mutagenesis with hydroxylamine and insertion of mutagenized DNA via transformation into wildtype A. quadruplicatum will be attempted.

Approach: See objective.

Progress: 87/01 to 87/12. The structure and composition of the phycobilisomes of wild type Agmenellum quadruplicatum strain PR-6 and a mutant lacking phycocyanin subunit genes were compared. The major phycobiliproteins are phycocyanin (PC) and allophycocyanin (AP), each composed of equimolar alpha and beta subunits. From spectroscopic and gel staining measurements the molar ratio of PC to AP was 2.0. PC is associated with bound linker polypeptides of 9, 29, and 33 kDa. AP is associated with linkers of 8.5 and 94 kDa, the latter being a phycobiliprotein as well. Also associated with AP are two minor phycobiliproteins, one AP beta-like and one called APB alpha. Amino terminal amino acid sequences (approximately 20 residues each) were determined for purified 29, 33 & 94 kDa linker polypeptides, as well as the AP beta-like polypeptide. By electron microscopy, isolated phycobilisomes of PR-6 have a hemidiscoidal shape typical of those from cyanobacteria. A mutant of PR-6 lacking genes for both subunits of PC was generated by use of a plasmid clone containing PC subunit genes and flanking sequences; the two adjacent PC alpha and beta genes were excised and replaced with a gene encoding neomycin phosophotransferase, which confers resistance to kanamycin in PR-6. This clone was transformed into PR-6 under selection for kanamycin. Isolation of phycobiliproteins from this mutant showed a complete lack of PC while AP was present at reduced levels compared to wild type.

Publications: 87/01 to 87/12

BUZBY, J.S., PDRTER, R.D. and STEVENS, S.E., JR. 1983. Plasmid transformation in "Agmenellum quadruplicatum", strain PR-6; construction of biphasic plasmids and characterization of their transformation properties.

DE LDRIMIER, R., BRYANT, D.A., PDRTER, R.D., LIU, W.Y., JAY, E. and STEVENS, S.E., JR. 1984. Genes for the alpha and beta subunits of phycocyanin. Proc. Nat'l. Acad. Sci. (USA) 81:7946-7950.

BRYANT, D.A., DE LDRIMIER, R., LAMBERT, D.H., DUBBS, J.M., STIREWALT, V.L., STEVENS, S.E., JR., PDRTER, R.D., TAM, J., and JAY, E. 1985.

BRYANT, D.A., DE LDRIMIER, R., PDRTER, R.D., LAMBERT, D.H., DUBBS, J.M., STIREWALT, V.L., FIELDS, P.I., STEVENS, S.E., JR., LIU, W.Y., TAM, J., JAY, E.W.K. 1985. Phycobiliprotein genes in cyanobacteria and cyanelles.

STARNES, S.M., LAMBERT, D.H., MAXWELL, E.S., STEVENS, S.E., JR. and PORTER, R.D. 1985. Contranscription of the large and small subunit genes of ribulose-1,5-bisphosphate carboxylase/oxygenase in "Cyanophora paradoxa." LAMBERT, D.H., BRYANT, D.A., STIREWALT, V.L., DUBBS, J.M., STEVENS, S.E., JR.

and PDRTER, R.D. 1985. Gene map for the "Cyanophora paradoxa" cyanelle genome. J. Baceriol. 164:659-664.

67.089 CRISO095293 MOLECULAR SWITCHES IN PLASTID DIFFERENTIATION

BDYER C D; HARDISDN R C; EVENSEN K B; Horticulture; Pennsylvania State University, University Park, **PENNSYLVANIA** 16802. Proj. No.: PENO2782 Project Type: HATCH Agency ID: CSRS Period: O1 MAR 85 to 30 JUN 89

Objectives: To compare genome organization and gene expression in different plastid types from the same species; to investigate gene products important in plastid differentiation.

Approach: Isolated intact chloroplasts, chromoplasts and amyloplasts of high purity will be used. The plastid genomes will be compared by restriction enzyme analysis and blot hybridization. Genome libraries of plastic DNAs will be constructed. Gene expression will be studied in isolated plastids by in vitro translation of plastid mRNAs and hybridization of plastid RNAs to the plastid genomic clones. Plastid gene products will be examined by two dimensional gel electrophoresis of 35S-methionine labeled peptides produced from feeding isolated plastids. Nuclear gene products will be examined from 35S-methionine labeled peptides obtained from plastids isolated from chloramphenicol or streptomycin treated tissue.

Progress: 88/01 to 88/12. A rapid method for the isolation of squash ('Cucurbita pepo' L.) chloroplast DNA (cpDNA) was developed using the methods of Gounaris et al. (Current Genet., 12:219, 1988). Restriction enzyme analysis of cpDNA with SalI, PvuII, BqlI, SacII and PstI resulted in fragments with a combined size of 166 kb. Large fragments were estimated from subfragments in second digestions. Preliminary characterization of fruit chromoplast DNA with PstI showed the same fragment pattern as obtained with chloroplast DNA. Work is in progress to map the squash plastid genome. Total RNA was isolated from tomato fruit at various stages of fruit ripening. This RNA was subjected to Northern blot analysis with probes from cloned tomato cpDNA PstI fragments cut with EcoRI and randomly labelled. Chloroplast RNA transcripts were detected through the red ripe stage of Traveler 76 grown under greenhouse conditions. Dnly at the overripe stage was a large decline in transcripts observed. These results differ from the previously reported rapid decline of chloroplast transcripts during the ripening of cherry tomatoes (Gruissem and coworkers). These differences are under investigation.

Publications: 88/01 to 88/12 ND PUBLICATIONS REPDRTED THIS PERIDD.

67.090* CRISO134836 STRUCTURE AND FUNCTION OF SELF-INCOMPATIBILITY GENES: A BIOTECHNOLOGICAL APPROACH

FLORES H; KAO T H; Plant Pathology; Pennsylvania State University, University Park, PENNSYLVANIA 16802.

Proj. No.: PENO2997 Project Type: HATCH Agency ID: CSRS Period: O1 JUL 88 to 30 JUN 91

Objectives: Isolate and sequence the S-genes coding for the S-proteins of the gametophytic self-incompatibility system in three genera of the Solanaceae family; identify the regulatory elements of these S-genes which control their tissue-specific expression; transfer a functional S-gene into a self-compatible mutant to test whether the mutant's behaviors can be reversed back to the wild-type self-incompatible; develop an in vitro transformation and flowering system to study the expression and function of the S-gene.

Approach: Appropriate laboratory procedures will be used to accomplish the stated objectives.

Progress: 88/07 to 88/12. We have established optimal conditions for the 'in vitro' culture of tissue from 'Nicotiana alata' and 'Petunia inflata'. Using the standard Murashige and Skoog medium, supplemented with benzyl-adenine (1 mg/ml), we regenerated plantlets from leaf disc explants. This system is now being used to raise transgenic plants, with a view to studying the molecular biology of the self-incompatibility genes (S alleles). A cDNA clone of the Sz allele of 'N.alata' was ligated into the vector pBI121, 3' to a CaMV S35 promoter and 5' to GUS, a reporter gene. This construct was introduced into 'Agrobacterium tumefaciens' (LBA4404) by triparental mating, and the bacteria then used to infect 'N.tabacum' leaf discs. Shoots were regenerated on selection plates (the plasmid confers kanamycin resistance) and are being tested for integration of Sz into the host genome. 'Agrobacterium' containing pBI121, and pBI121::Sz, was also used to infect leaf discs of 'N.alata' bearing another S allele (S(f11)). Another vector, pPCV702, which lacks the GUS gene but contains the CAMV \$35 promoter and confers kanamycin resistance, is being used to raise transgenic plants. The cDNA clone of the 'Petunia' S(2) allele was cloned into this vector, and transformants containing 'sense' and 'anti-sense' configurations of the clone are being identified. 'Agrobacterium' containing these constructs will be used to infect 'Petunia' leaf discs.

Publications: 88/07 to 88/12 NO PUBLICATIONS REPORTED THIS PERIOD.

67.091 CRISO133187 GENETIC MANIPULATION OF PROTEIN IMPORT INTO PLANT MITOCHONDRIA

CASHMORE A; Office of Research Admin.; University of Pennsylvania, Philadelphia, PENNSYLVANIA 19104.

Proj. No.: PENR-8700935 Project Type: CRG0 Agency ID: CRG0 Period: 01 AUG 87 to 31 JUL 88

Objectives: PROJ. 8700935. To define the mechanism by which proteins are imported into plant mitochondria.

Approach: A cDNA sequence for the mitochondrial enzyme citrate synthase has been isolated. Chimeric constructs will be made from this sequence and the gene for the small subunit of

ribulose-1,5-bisphosphate carboxylase. This latter polypeptide is normally localized in chloroplasts. In vitro import studies will be carried out to define the peptide regions that distinguish import into mitochondria and chloroplasts.

67.092 CRISO096739 ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA

MULLIN B C; Knox County; University of Tennessee, Knoxville, **TENNESSEE** 37996. Proj. No.: TENR-8500727 Project Type: CRGD Agency ID: CRGO Period: O1 AUG 85 to 31 JAN 87

Objectives: Proj. 8500727. The primary objective of this study is to determine whether DNA sequences in Alnus glutinosa that hybridize to a soybean leghemoglobin probe are in fact related to leghemoglobin genes in soybean.

Approach: Hybridizing sequences from Alnus glutinosa will be cloned and sequenced and the extent of homology with known soybean leghemoglobin coding sequences will be determined.

Progress: 87/01 to 88/01. No progress reported this period.

Publications: 87/01 to 88/01
 DURKEE, S.M. and MULLIN, B.C. A truncated
 globin gene in Alnus glutinosa. Submitted.

67.093 CRISO136133 REGULATION OF PLANT GENE EXPRESSION BY CELL TURGOR OR ABSCISIC ACID

MULLET J E; Biochemistry & Biophysics; Pb Box 3578, College Station, **TEXAS** 77843.

Proj. No.: TEXO6951 Project Type: CRGO Agency ID: CRGO Period: O1 AUG 88 to 31 JUL 90

Objectives: PROJ. 8801234. To identify genes which are differentially induced or repressed in plant cells when turgor is decreased in water deficient plants. To delineate the signal transduction pathway which connects altered cell turgor and gene expression.

Approach: Differential cDNA clone screening will be used to identify turgor--or ABA-responsive genes. cDNAs will be characterized by sequence analysis. cDNA probes will be used to determine if changes in gene expression are due to changes in transcription. Cis and trans-acting elements which regulate gene expression will be characterized.

Progress: 88/08 to 88/12. This is a new project therefore only a brief report is submitted. cDNAs to four-turgor responsive genes were obtained and a preliminary characterization carried out. The genes were sequenced and no similar sequences were found in the Intelligenetics data bank. One ABA responsive cDNA clone was isolated from pea.

Publications: 88/08 to 88/12
GUERRERO, F. and MULLET, J.E. 1988. Reduction
 of Turgor Induces Rapid Changes in Leaf
 Translatable RNA. Plant Physiol.
 88:401-408.

67.094* CRISO034131 REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO

PARK W D; Biochemistry & Biophysics; Texas A&M University, College Station, **TEXAS** 77843. Proj. No.: TEXO6769 Project Type: CRGO Agency ID: CRGO Period: 15 SEP 83 to 30 JAN 86

Objectives: Project 8300489. In this project we will look in more detail at the regulation of the major tuber protein genes. cDNA clones will be used to examine the metabolism of the tuber protein mRNAs during normal tuber development and also in other tissues such as stems and leaves under both inducing and noninducing conditions.

Approach: The genes for patatin will be isolated by cloning in lambda phage vectors and their structures will be determined. The relationship of gene structure and function will then be examined by looking at DNAse I sensitivity and methylation patterns.

Progress: 83/09 to 86/12. We have isolated and characterized a number of genomic clones of the major potato tuber protein, patatin. These clones consist of two types, one of which (Class II), contains a 22 bp insertion in the 5' untranslated region. The genomic clone pPS20, which does not contain the insert (Class I), has been completely sequenced. It contains 6 introns and codes for an mRNA that is identical to our previously characterized cDNA clone pGM203. The 5' flanking region of three other genes without inserts has also been examined and found to be highly homologous. Genes without the insertion are expressed in tubers and can be induced to be expressed in stems and petioles, but are not normally expressed in roots. The first two Class II genes that we examined appear to be pseudogenes since they contain stop condons and we are unable to get complete protection in \$1 experiments. However, based on primer extension experiments, we have found that insert containing mRNAs are expressed tubers and, at a lower level, in roots. Interestingly, we have also found patatin related sequences in tomato. These appear to be Class II genes since they are expressed in roots and contain the 22 bp insertion.

Publications: 83/09 to 86/12

HANNAPEL, D.J., MILLER, J.C., Jr., and PARK,
W.D. Regulation of Potato Tuber Protein
Accumulation by Gibberellic Acid. Plant
Physiol. 78, 700-703. (1985).

HANNAPAL, D.J. 1985. Hormonal Regulaton of
Tuber Protein Synthesis. Ph.D.

Thesis, Purdue University.

PIKAARD, C.S. 1985. Molecular
Characterization of the Patatin Gene Family
of Potato. Ph.D. Thesis, Purdue University.

PIKAARD, C.S., MIGNERY, G.A., DIN POW MA, STARK, V.J. and PARK, W.D. 1986.

Sequence of Two Apparent Pseudogenes of the Major Potato Tuber Protein, Patatin. Nucleic Acids Research 4:5564-5566.

BOURQUE, J.E., MILLER, J.C. and PARK, W.D. 1987. Use of an In Vitro Tuberization System to Study Tuber Protein Gene Expression. In Vitro (in press).

PIKAARD, C.S., BURSCA, J.S., HANNAPEL, D.J., MIGNERY, G.A. and PARK, W.D. The Major Potato Tuber Protein, Patatin, is Expressed in Roots: Root Transcripts Contain a 22 Nucleotide Insertion.

67.095 CRISO034192 REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO

PARK W D; Biochemistry & Biophysics; Texas A&M University, College Station, **TEXAS** 77843. Proj. No.: TEX-8100232-1(6768)

Project Type: CRGO Agency ID: CRGO Period: 01 JUL 84 to 30 JUN 85

Objectives: PROJ. 8100232-1. To examine the regulation of storage tissue differentiation in potatoes, we are studying the regulation of the major tuber protein, patatin. Patatin accounts for approximately 40% of the soluble protein in mature tubers, but neither patatin or its mRNA are normally present in significant amounts in stolon tips from noninduced plants or in leaves, stems or petioles from either induced or noninduced plants. However, if tubers or other alternate sinks are removed, patatin can accumulate in large amounts in stems and petioles. This accumulation can occur without tuber-like swelling and will occur under conditions normally noninductive for tuberization, but is accompanied by the accumulation of the other major tuber proteins and large amounts of starch.

Approach: Protein and nucleic acid biochemistry.

Progress: 85/01 to 85/12. The major tuber protein patatin has attracted a great deal of interest both as a biochemical marker for tuberization and as a tuber-specific promoter for gene transfer studies, but we have found that the structure and regulation of the patatin multigene family is surprisingly complex. While patatin is not normally present in the stems and leaves of early season cultivars such as Superior, under certain conditions it can be induced to accumulate in massive amounts in stems and petioles. We have also found that the level of patatin in stem and leaves under normal field conditions varies among cultivars and is apparently related to their maturity group classification. In addition to tuber and stem forms of patatin, we have found that all cultivars contain an immunologically distinct form of patatin in roots. The root form is encoded by a highly homologous mRNA, but has a different developmental patter. The patatin in tubers, roote, and that normally present in stems differ in their 2-D gel profile and may be the products of different genes based on differences in the \$1 nuclease protection of 5'

patatin cDNA probes with tuber and root RNA and apparent differences in their sensitivity to gibberellic acid. Thus while patatin does appear to be a very favorable candidate both as a biochemical marker for somatic storage tissue differentiation and as a tuber specific promoter, the isoform that one works with will apparently be very crutical. A manuscript describing these results is currently in preparation.

Publications: 85/01 to 85/12 MIGNERY, G.A., PIKAARD, C.S., HANNAPEL, D.J. and PARK, W.D. Isolation and sequence analysis of cDNAs for the Major Potato Tuber Protein, Patatin. Nucleic Acids Research 12, 7987-8000. (1984). HANNAPEL, D.J., MILLER, J.C., JR. and PARK, W.D. Regulation of Potato Tuber Protein Accumulation by Gibberellic Acid. Plant Physiol. 78, 700-703 (1985). HANNAPEL, D.J. 1985. Hormonal Regulation of Tuber Protein Synthesis. Ph.D. Thesis, Purdue University. PIKAARD, C.S. 1985. Molecular Characterization of the Patatin Gene Family of Potato Ph.D. Thesis, Purdue University. PARK, W.D., HANNAPEL, D.J., MIGNERY, G.A. and PIKAARD, C.S. Molecular Approaches to the Study of the Major Tuber Proteins. IN: Potato Physiology, Paul, H.L. (ed.), Academic Press, (1985) pp. 261-278.

67.096 CRISO096199 CELL SPECIFIC GENE EXPRESSION DURING LATICIFER DIFFERENTIATION IN POPPY

NESSLER C L; Biology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX-8500095 Project Type: CRG0 Agency ID: CRG0 Period: 15 JUL 85 to 31 JUL 88

Objectives: PROJ 8500095. The objectives of this program include the isolation, and cloning of genes which are uniquely expressed in laticifers of the opium poppy, Papaver somniferum. Emphasis will be directed toward identifying the genes which code for enzymes of the morphinan alkaloid synthetic pathway. Specific goals of this project include: Identification of gene products expressed during laticifer differentiation using two dimensional gel electrophoresis and production of antisera against laticifer specific proteins. Construction of cDNA libraries from polyadenylated RNAs from latex in the bacteriophage expression vector gt 11.

Approach: Screening of the cDNA libraries from latex using plaque hybridization using single stranded cDNA from leaf mesophyll cells and callus cultures as a probe and thereby identify and isolate genes encoding laticifer specific mRNAs. Production of fusion polypeptides by laticifer specific cDNA clones and their screening with the antisera prepared against latex specific proteins produced in 1.

Progress: 87/01 to 87/12. Although there is at least one report in the literature that opium poppy does not infect with wild-type A. tumifaciens, the primary reference in this

review is old (1924) and apparently only one bacterial strain was tested. It is not surprising, however, that poppy might not form tumors with wild-type Agrobacterium since we have found that the auxin:cytokinin ratio needed for optimal callus initiation and growth in poppies is different from that of tobacco or other gall forming species. Since disarmed vectors do not depend on tumor formation, callus growth should be strictly a function of the hormonal composition of the medium and antibiotic resistance conferred by the T-DNA. Preliminary data from our laboratory indicates that opium poppy can be successfully transformed by the binary Agrobacterium vector system. Our initial transformation attempts have been made using the very strong constitutive promoter from cauliflower mosaic virus, CaMV 35S, driving expression of the GUS coding region in the T-DNA of Bin19. The reporter gene used in these studies (GUS) codes for the bacterial enzyme Beta-glucuronidase (GUS, EC.3.2.1.31) and was kindly provided by Richard Jefferson of the Plant Breeding Institute, Cambridge, England. When incubated in the chromogenic substrate, 5-bromo-4-chloro-3-indolyl beta-D-glucuronide (X-GLUC), cells expressing GUS turn blue. Transformed opium poppy callus, expressing GUS activity, have been observed in several independent experiments.

Publications: 87/01 to 87/12
NESSLER, C.L. 1988. Comparative analysis of
 the major latex proteins of opium poppy. J.
 Plant Physiol., in press.

67.097 CRISO034120 GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOID WHEAT AND ITS RELATIVES

HART G E; Soil & Crop Sciences; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEXO6777 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 83 to 31 DEC 86

Objectives: Proj 8300637. To determine in each of seven diploid Triticeae relatives of the cultivated wheats the chromosomal locations of loci that are homoeologous (related) to sets of isozyme structural gene loci located in each of the 14 homoeologous chromosomal arm groups in hexaploid wheat and to determine the genetic map locations of the members of a number of homoeologous sets of loci in the three genomes of hexaploid wheat and in three diploid Triticeae species.

Approach: The chromosomal locations of alien genes will be determined by analyzing the zymogram phenotypes produced by chromosome addition lines. The telocentric method and Mendelian studies will be used to determine the genetic map locations of genes.

Progress: 83/09 to 86/12. The objectives of this project are to identify and determine the chromosomal locations of homologous unique sequence gene loci in genomes in the tribe Triticeae and to investigate the genetic organization and evolutionary relationships among Triticeae chromosomes and genomes. Enzyme

structural genes have been studied for several years. DNA research is now being initiated, the loci to be studied being those that show allelic variation in restriction fragment length. Four putative wheat-T. searsii disomic chromosome addition lines have been identified and a number of genes localizedin T. searsii chromosomes. The chromosomal locations of Skdh-1 loci were determined in several Triticeae species. The intra-chromosomal map location of Ndh-B1 was determined and studies of the map locations of the Aco-1 and Aco-2 loci were continued. Studies of the tissue and developmental specificity of glucosephosphate isomerase revealed that one homologuous set of genes located at the Gpi-1 locus is differentially expressed in the embryo versus other tissues while the second set of Gpi-1 genes is active in all of the tissues examined. Further progress was made in developing a complete set of wheat-T. longissimum disomic chromosome addition and substitution lines.

Publications: 83/09 to 86/12

BENEDETTELLI, S., and HART, G.E. 1986. Further evidence regarding the genetic control of Triticum aestivum glucosephosphate isomerase-1. Genetics 113 (1, pt. 2):s43.

BENEDETTELLI, S., and HART, G.E. 1986. Genetic analysis ofTriticeae shikimate dehydrogenase. Agronomy Abstracts, p. 57

HART, G.E., GALE, M.D. 1986. Proposed "Guidelines for Nomenclature of Biochemical Loci in Wheat and Related Species." Wheat Newsletter 32:19-20.

HART, G.E., and GALE, M.D. 1986. Gene Symbols for Wheat Proteins, 1986 Supplement. Wheat Newsletter 32:211-214.

HART, G.E. and GALE, M.D. 1986. Nucleolar Organizer Regions and Proteins. In: Catalogue of Gene Symbols for Wheat, 1986 Supplement. McIntosh, R.A. (Ed.). Wheat Newsletter 32:203-205.

HART, G.E, and GALE, M.D. 1987.
Biochemical/molecular loci of hexaploid wheat

(In press). In: Genetic Maps: Vol. 3.
 O'Brien, S.J. (Ed.). Cold Spring Harbor
Laboratory.

NEUMAN, P.R., and HART, G.E. 1986. Genetic Control of the Mitochondrial Form of Superoxide Dismutase in Hexaploid Wheat. Biochem. Genet. 24:435-436.

67.098 CRISO034172 BACTERIAL GENES CODING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE

TABITA F R; Microbiology; University of Texas, Austin, **TEXAS** 78712.

Proj. No.: 8300705 Project Type: CRGD Agency ID: CRGO Period: 01 SEP 83 to 31 AUG 88

Objectives: Proj 8300705. Determine the location of the genes (whether they are chromosomal or plasmid-borne) encoding the two distinct RubisCO enzymes of Rhodopseudomonas sphaeroides. Determine how the activity of RubisCO might be regulated in vivo.

Approach: The general approach to objective 1 will be to use available probes to determine the location of RubisCO structural genes. In objective 2, immunological procedures will be used to verify that Form II RubisCO is constitutive and thus subject to some form of activation in vivo.

Progress: 88/01 to 88/12. The form II ribulose bisphosphate carboxylase/oxygenase (RubisCO) gene from Rhodobacter sphaeroides had been previously cloned in this laboratory. More recently, we isolated the large subunit and small subunit genes of the form I RubisCO. Both RubisCO genes were sequenced by the Sanger chain termination method. The deduced amino acid sequence of the large subunit of the form I enzyme was highly homologous to the plant large subunit. Indeed many of the enzymatic properties of this plant-type bacterial protein resemble the properties of the plant enzyme. The deduced amino acid sequence of the form II large subunit showed little homology to the form I protein, perhaps correlating with differences in function. Both the form I and form II RubisCO genes were expressed in Escherichia coli and the recombinant proteins shown to be identical to the enzymes isolated from Rhodobacter, suggesting that these recombinant proteins might be utilized for structure-function studies.

Publications: 88/01 to 88/12
WAGNER, S. J., S. E. STEVENS, JR., B. T.
NIXON, D. H. LAMBERT, R. G. QUIVEY, Jr.,
and F. R. TABITA. (1988). Nucleotide and
deduced amino acid sequence of the
Rhodobacter sphaeroides gene encoding form
II ribulose bisphosphate carboxylase/o.

67.099 CRISO138161 A PLANT MITOCHONDRIAL MATURASE GENE

WOLSTENHOLME D R; University of Utah, Salt Lake City, **UTAH** 84112.

Proj. No.: UTAR-8900674 Project Type: CRGD Agency ID: CRGO Period: O1 JUN 89 to 31 MAY 91

Objectives: PROJ. 8900674. The work proposed is designed to gain information on the structure and transcription of a maturase (mat) gene contained in an intron of the respiratory chain NADH dehydrogenase subunit 1 (nadi) gene in corn and soybean mtDNAs, and on whether these mtDNAs contain multiple nadi genes with different intron-exon arrangements.

Approach: Experiments involving DNA sequencing, DNA:DNA hybridization, and sequence analysis of RNA transcripts using reverse transcriptase will be carried out to determine the exact interrelationships of the mat gene-containing intron and the nadi gene exons, whether multiple nadi genes with different intron-exon arrangements occur in soybean and corn mtDNAs, and structural details of the nadi genes. Northern blot analysis will be used to seek evidence of transcription of corn and soybean mat genes. Further nucleic acid hybridization experiments will be carried out to map mat gene transcripts on mtDNA molecules and to determine details of processing of these transcripts.

Whether the corn mat gene-containing intron is self-splicing in vitro will be tested using run off transcripts of this intron that have been cloned in an SP6 vector.

67.100 CRISO132263 PHYSIOLOGIC AND GENETIC STUDIES OF APOMIXIS IN ELYMUS RECTISETUS

CARMAN J G; Plant Science; Utah State
University, Logan, **UTAH** 84322.
Proj. No.: UTAO0313 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 87 to 30 JUN 92

Objectives: Cytochemically compare ovular development prior to, during, and after sexual and apomictic megasporogenesis; employ Giemsa banding and in situ hybridization to cytologically karyotype each E. rectisetus chromosome; adapt isozyme procedures and isolate RFLP's for use as genetic markers of E. rectisetus chromosomes; determine linkage relationships between isozymes and RFLP's; conduct a genetic analysis of apomixis in E. rectisetus and identify linkage relationships between markers and apomixis genes.

Approach: Procedures to be used include cytohistochemical staining for callose, cellulose, hemicellulose and protein; transmission electron microscopy for microtubules, microfibriles, dictyosomes, mitochondria and rough and smooth endoplasmic recticulum; giemsa C and N banding; RFLP and isozyme analyses; standard linkage procedures for sexual E. scabrus; and backcrossing and introgressive hybridization for genetic analysis of apomixis.

Progress: 88/01 to 88/12. The objective of this project is to transfer apomixis (asexual reproduction through seed) from Elymus rectisetus to wheat through wide-hybridization and backcrossing. This would permit the production of F1 hybrid wheats that reproduce themselves by seed. Because of yield advantages, farmers could maintain current production levels with a 10 to 20% reduction in land, energy and human inputs. Apomixis would also reduce the high cost of producing hybrid wheat seed. During 1988 we made several important interspecific and intergeneric hybrids: wheat x E. rectisetus and Pseudoroegneria spicata x E. rectisetus. The former hybrids died as embryos. The latter hybrids are vigorous and are important because they may be used as bridging germplasm in the transfer of apomixis. We have also made considerable progress in the tissue culture of wheat and E. rectisetus such that it might now be reasonable to obtain hybrids between wheat and the apomict by somatic cell hybridization followed by plant regeneration. Success at this would eliminate the need for intergeneric hybridization by crossing. Thus, research during 1988 focused largely on cell culture of wheat and apomictic E. rectisetus.

Publications: 88/01 to 88/12 CARMAN, J.G., JEFFERSON, N.E., and CAMPBELL, W.E. 1988. Induction of embryo-genic Triticum aestivum L. calli. I. Quantification of genotype and culture medium effects. Plant Cell Tissue Organ Cult. 12:83-95. CARMAN, J.G., ET AL. 1988. Induction of embryo-genic Triticum aestivum L. calli. II. Quantification of organic addenda and other culture variable effects. Plant Cell Tissue Organ Cult. 12:97-110. CARMAN, J.G. 1988. Improved somatic embryogenesis in wheat by partial simulation of the in-ovulo oxygen, growth-regulator and desiccation environments. Planta 175:417-424. CARMAN, J.G. 1988. Improving in vitro somatic embryogenesis by simulating in ovulo environments. 39th Annual Mtg., Tissue Culture Assoc., June 12-15. Las Vegas, NV. In Vitro 24:71A. CARMAN, J.G. 1988. Enhancing somatic embryogenesis in wheat by pretreatments that alter hormone levels during explant differentiation. Annual Mtg., Amer. Soc. of Agronomy, Agron. Abs. p. 166.

67.101 CRISO095796 EXPLOITATION OF RIBOSOMAL DNA TO CONTROL PLANT PATHOGENIC FUNGI

CARMAN, J.G. 1988. The in-ovulo environment

and its relevance to cloning wheat via

somatic embryogenesis. In Vitro (in press).

ULLRICH R C; Botany; University of Vermont, Burlington, VERMONT 05405.

Proj. No.: VT-B0-00404 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To define the structure and organization of the rDNA unit repeat in Schizophyllum.

Approach: These studies use nucleic acid techniques to examine the structure of the rDNA unit repeat. This DNA has been cloned, and a restriction map will be completed. RNA-DNA hybridizations will be used to locate the rRNA cistrons and the promoter region. S(1) nuclease studies will be used to locate the initiation sequence for transcription, and DNA sequencing will reveal the nucleotide structure of the DNA. Analogies of Schizophyllum rDNA to those in wheat, Xenopus and Drosophila suggest that the rDNA may be used to control plant pathogenic fungi.

Progress: 88/01 to 88/12. NO PROGRESS REPORTED THIS PERIOD

Publications: 88/01 to 88/12

BUCKNER, B., and ULLRICH, R.C. 1988.

Developmental regulation of the methylation of the ribosomal DNA in the basidiomycete fungus, Schizophyllum commune. Am.

Inst. of Biol. Sci., D

BUCKNER, B., C.P. NOVOTNY, and R.C. ULLRICH 1989. Ribosomal

BUCKNER, B., NOVOTNY, C.P., and ULLRICH, R.C. 1988. Organization of the ribosomal RNA genes of Schizophyllum commune. Cu

BUCKNER, B., NOVOTNY, C.P., and ULLRICH, R.C. 1988. Developmental regulation of the methylation of the ribosomal DNA in the basidiomycete fungus Schizophyllum commune. Curr. Genet.

CHASE, T.E., and ULLRICH, R.C. 1988. Heterobasidion annosum, root and butt-rot of trees. Pages 501-510 in G.S. Sidhu (ed.). Advances in plant pathology, vol.

6, genetics of pathol

CHASE, T.E., and ULLRICH, R.C. 1988. Heterobasidion annosum, root- and butt-rot of trees. Intl. Union Forest Res. Org. Conf. Root & Butt Rots Forest Trees, Vernon, B.C., Canada. A

ULLRICH, R.C., and ANDERSON, J.B. 1988. Armillaria mellea, cause of rots in woody species. Pages 491-499 in G.S. Sidhu, ed (eds.). Advances in Plant Pathology, vol. 6, Genetics of Pathological Fungi. Academic Press, London, England.

67.102* CRISO136218 ISOLATING AND CHARACTERIZING A-BETA MATING-TYPE ALLELES OF SCHIZOPHYLLUM COMMUNE.

ULLRICH R C; Botany; University of Vermont, Burlington, VERMONT 05405. Proj. No.: VT-B0-00442 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: Examine structure and function of A-beta mating-type alleles from the basidiomycete, Schizophyllum commune. Compare the structure of two or more wild-type A-beta alleles and that of an A-betal constitutive mutant allele. Define functional domains of the A-beta locus.

Approach: Isolate A-beta alleles from cosmid libraries. Subclone cosmid inserts isolated to smallest functional units active in transformation. Obtain and analyze DNA sequence. Define functional domains of A-beta locus by in vitro mutagenesis and transformation. Determine if transcript is made from A-beta locus.

Progress: 88/01 to 88/12. This project has been active for only a few weeks.

Publications: 88/01 to 88/12

DRUMMOND, B.J. 1988. Analysis of TRP1 in wild-type, trp mutant and trpt transformant strains of Schizophyllum commune. M

GIASSON, L., MILGRIM, C., SPECHT, C.A. NOVOTNY, C.P., and ULLRICH, R.C. 1988.

- A multiallelic mating-type alleles of the basidiomycete, Schizophyllum commune. Genome 30:300. Abstr.
- PHELPS, L., BURKE, J., ULLRICH, R.C., and NOVOTNY, C.P. 1989. Nucleotide base sequence of the mitochondrial COIII gene o
- SPECHT, C.A., MUNOZ-RIVAS, A.M., NOVOTNY, C.P., and ULLRICH, R.C. 1988. Transformation of Schizophyllum commune: an analysis of parameters for improving transformation frequencies.

SPECHT, C.A., MUNOZ-RIVAS, A.M., NOVOTNY, C.P., and ULLRICH, R.C. 1989. Transformation of Schizophyllum commune: an anna

- ULLRICH, R.C., L. GIASSON, C. MILGRIM, C.A. SPECHT, C.P. NOVOTNY 1988. Isolating mating-type genes of the wood-rotting Basidiomycete and pathogen Schizophyllum commune. Proc. Intl.
- ULLRICH, R.C., L. GIASSON, C. MILGRIM, C.A. SPECHT, and C.P. NOVOTNY 1988. Cloning and analysis of A mating-type alleles from Schizophyllum commune. Mating type control in lower e
- ULLRICH, R.C., GIASSON, L., MILGRIM, C., SPECHT, C., NOVOTNY, C.P. 1988. Isolation of alpha mating-type genes and directed alteration of mating type of.

67.103 CRISO085751 A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIDIUM PASTEURIANUM AND OTHER **MICROORGANISMS**

JOHNSON J L; CHEN J S; Anaerobic Microbiology; Virginia Poly Inst, Blacksburg, VIRGINIA 24061.

Proj. No.: VA-6124400-018 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 81 to 30 SEP 86

 $\begin{array}{lll} \textbf{Objectives:} & \textbf{Study the following aspects of} \\ \textbf{N(2)-fixing genes in Clostridium pasteurianum,} \end{array}$ Azospirillum lipoferum and Azospirillum brasilense: The organization of nif structural genes, nucleotide sequence of nif H gene, its control region and codon usage, and kinetics of synthesis and degradation of mRNA for nitrogenase.

Approach: The nif structural genes will be cloned into pBR322 plasmid and th organization determined by restriction nuclease mapping and by nucleotide sequencing. The kinetics of mRNA turnover will be measured by hybridizing labeled cloned DNA with RNA isolated from repressed and derepressed cells.

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Progress: 85/01 to 86/09. The protein components of the nitrogenase complex are coded for by three structural genes: nifH for the Fe protein and nifD and nifK for the MoFe protein. We are studying (1) the nif genes of Clostridium pasteurianum by cloning and nucleotide sequence analysis, and (2) the structure-function relationship of nitrogenase components by comparing nifHDK-encoded sequences of different organisms. We have cloned and completed nucleotide-sequencing of the nitrogenase structural genes of C. pasteurianum as well as a portion of nifE, which is involved in the formation of active MoFe protein. The genes occur in the order of nifH1DKE. nifH1 codes for the Fe protein, and the nifH1-encoded amino acid sequence is identical to the sequence obtained from protein. nifD and nifK overlap in this organism, which may be a regulatory feature pertaining to translational efficiency. The amino acid sequences deduced from nifD and nifK largely agree with sequences previously determined with the protein. The DNA-derived sequences are expected to be the correct ones. Among the nitrogenase components, the only posttranslational processing involving peptide bonds is the removal of a methionine residue from the nifD-encoded polypeptide. In addition

to nifH1, this organism contains five nifH-like sequences (nifH2 through nifH6). nifH2, H3, H4, and part of H5 have been sequenced. The nifH2-and H4-encoded amino acid sequences are over 90% homologous with the Fe protein sequence. Possible functions of these nifH-like sequences will be studied next.

Publications: 85/01 to 86/09

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WANG, S.-Z. and CHEN, J.-S. and JOHNSON, J. L., 1986, Two nitrogenase structural genes (nifD and K) overlap in the anaerobe (Clostridium pasteurianum W5)., Federation Proc. 45:1634.

67.104 CRISO098061 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY

KLEINHOFS A; Agronomy & Soils; Washington State University, Pullman, WASHINGTON 99164. Proj. No.: WNPO0745 Project Type: HATCH Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To isolate and to characterize genes in plants controlling nitrogen metabolism, carbon metabolism, development and resistance to pathogens.

Approach: Barley nitrate reductase will be cloned from mRNA enriched by size fractionation. The cDNA clone will be identified by immunological screening in a lambda gt 11 expression system. The cDNA clone will be used to select genomic clones. These will be characterized by restriction mapping. RNA transcript mapping and eventually sequencing. Expression of the cloned genes will be studied by transfer to nitrate reductase-deficient tobacco mutants. The gene transfer will be by Ti mediated vectors or by electroporation.

Progress: 88/01 to 88/12. The barley NADH nitrate reductase cDNA clone bNRp10 was used as a hybridization probe to map the homologous gene to the short arm of barley chromosome 6H, rye chromosome 4R, Aegilops umbellulata chromosomes 6U and 7U and the short arm of wheat chromosomes 6A, 6B, 6D, 7A, 7D and long arm of chromosome 4B. More detailed mapping showed that the nitrate reductase loci are distal to the ribosomal RNA loci in both barley chromosome 6H and wheat chromosome 6B, but that the genetic distances and arrangement with respect to other loci were different.

Publications: 88/01 to 88/12
BLAKE, T.K. and KLEINHOFS, A. 1988.
Applications of molecular genetics to crop improvement. In: R.J. Summerfield, ed., World Crops: Cool Season Food Legumes.
MELZER, J.M., KLEINHOFS, A., KUDRNA, D.A., WARNER, R.L. and BLAKE, T.K. 1988. Genetic mapping of the barley nitrate reductase-deficient nar1 and nar2 loci.

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OWAIS, W.M. and KLEINHOFS, A. 1988. Metabolic activation of the mutagen azide in biological systems. Mutation Research.
197:313-323.

KLEINHOFS, A., CHAO, S. and SHARP, P.J.
Mapping of nitrate reductase genes in
barley and wheat. In: Proc. Seventh
International Wheat Genetics Symposium,
Cambridge (in press).

67.105 CRISO033949 GENETICS OF NITRATE REDUCTION IN BARLEY

KLEINHOFS A; WARNER R L; Agronomy & Soils; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPOO605 Project Type: CRG0 Agency ID: CRG0 Period: O1 SEP 82 to 31 AUG 86

Objectives: Proj. No. 8200398. Our long-term goal is to understand the genetic regulation of nitrate assimilation in crop plants. Such knowledge is essential for the eventual genetic engineering of crop plants for improved nitrogen use efficiency.

Approach: In this project, wer propose: to conduct an intensive search for new genes controlling nitrate reductase activity, to investigate in detail the function of these genes and to study the molecular structure of the wild type and mutant nitrate reductase proteins. These studies will provide new insight in the genetic control and biochemical mechanisms of nitrate assimilation.

Progress: 82/09 to 86/08. Thirty-one nitrate reductase-deficient mutants have been selected and analyzed in our laboratory. Six additional mutants included in the analysis were obtained from other laboratories. These mutants represent 7 different complementation groups designated nar 1 through nar 7. The genes nar 1 and nar 7 code for the NADH specific and the NAD(P)H bispecific nitrate reductase apoproteins, respectively. The genes nar 2 through nar 6 are involved with the molybdenum cofactor functions. Nar 2 was mapped to chromosome 7 in close linkage with lys 3. Nar 1 appears to be located on chromosome 4. The other genes have not yet been mapped.

Publications: 82/09 to 86/08

SOMERS, D.A., KUO, T.M., KLEINHOFS, A. and WARNER, R.L. 1983. Nitrate reductase-deficient mutants in barley. Immunoelectrophoretic characterization. Plant Physiology 71:145-149.

Plant Physiology 71:145-149.
SOMERS, D.A., KUO, T.M., KLEINHOFS, A.,
WARNER, R.L. and OAKS, A. 1983. Synthesis
and degradation of barley nitrate
reductase. Plant Physiology 72:949-952.

KUO, T.M., KLEINHOFS, A., SOMERS, D.A. and WARNER, R.L. 1984. Nitrate reductase-deficient mutants in barley: Enzyme stability and peptide mapping. Phytochemistry 23:229-232.

KLEINHOFS, A., WARNER, R.L. and NARAYANAN, K.R. 1985. Current progress towards understanding of the genetics and molecular biology of nitrate reductase in higher plants. In: B.J. Miflin, ed. Oxford Survey of Plant Molecular & Cell Biol

HARKER, A.R., NARAYANAN, K., WARNER, R.L. and KLEINHOFS, A. 1986. NAD(P)H bispecific nitrate reductase in barley leaves: Partial purification and characterization. Phytochemistry 25:1275-1279.

67.106 CRISO093863 REGULATION OF PLANT GENE EXPRESSION

AN G; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO0692 Project Type: HATCH Agency ID: CSRS Period: 01 SEP 88 to 31 AUG 91

Objectives: The primary objective is to understand the regulatory mechanisms of plant gene expression and the molecular basis of differentiation. A molecular level understanding of growth and development is essential in genetically engineering plants for improved agronomic traits.

Approach: Regulatory regions of several plant genes are being analyzed by generating in vitro mutants and testing the effects in transgenic tobacco.

We have continued Progress: 88/01 to 88/12. to study plant gene organization and regulation using both stable and transient assay systems in order to understand the molecular basis of plant development and differentiation. One of the T-DNA genes, the nopaline synthase (nos) gene, has been studied in detail. The promoter activity is developmentally-regulated in various vegetative and reproductive organs. Fine deletion analysis of the nos promoter indicated that the immediate upstream region is composed of three elements: two 8 bp repeat elements and a 10 bp potential Z-DNA forming sequence. It appears that the Z element is essential for promoter function and specficity, and that the repeat elements are either enhancers or positive activators. The further upstream region appears to be necessary for promoter specificity in reproductive organs. We have also studied the regulatory region of the Arabidopsis thaliana chlorophyll a/b binding protein (cab) gene which is a member of photosynthetic families. It was found that there are multiple sets of regulatory elements in the control region. One of these elements, ATACGTGT, is essential for light-dependent leaf specific expression of the gene. This sequence is also conserved in several photosynthetic promoters. We have also studied rice nitrate reductase genes. DNA sequences of the nitrate reductase genes were obtained.

Publications: 88/01 to 88/12

- HA, S.B. and AN, G. 1988. Identification of Upstream Regulatory Elements Involved in the Developmental Expression of the Arabidopsis thaliana cab1 Gene. Proc. Natl. Acad. Sci. (USA) 85:8017-8021.
- AN, G., COSTA, M.A., MITRA, A., HA, S.B. and MARTON, L. 1988. Organ-Specific and Developmental Regulation of the Nopaline Synthase Promoter in Transgenic Tobacco Plants. Plant Physiol. 88:547-552.

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- MITRA, A., CHOI, H.K. and AN, G. 1988. Structural and Functional Analysis of Arabidopsis thaliana Chlorophyll a/b Binding Protein (cab) Promoters. Plant Mol. Biol. (in press).
- AN, G., THORNBURG, R., JOHNSON, R., HALL, G. and RYAN, C.A. 1988. A Possible Role for 3' Sequences of the Wound-Inducible Potato Proteinase Inhibitor II-K Gene in Regulating Gene Expression.
- RYAN, C.A. and AN, G. 1988. Molecular Biology of Proteinase Inhibitors in Plants. Plant, Cell and Environment (in press).

67.107* CRISO131841 AMINO ACID METABOLISM AND THE RHIZOBIUM-LEGUME SYMBIOSIS

KAHN M L; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO4772 Project Type: CRGO Agency ID: CRGO Period: O1 AUG 86 to 31 MAY 89

Objectives: PROJECT 8601281. The long term objective is to determine the detailed mechanism and regulation of the nutrient exchange in Rhizobium-legume symbiosis. Specific objectives are to investigate the role of amino acid catabolism in symbiotic nitrogen fixation. This approach is based on a nitrogen carrier model for nutrient exchange between the symbionts proposed by the PI.

Approach: Specifically we propose to generate mutants of R. meliloti that have defects in amino acid catabolism, determine the enzymatic defect in these mutants and assess the effect of the mutations on symbiotic nitrogen fixation. Generate mutants of R. meliloti that have defects in intermediary carbon metabolism, determine the enzymatic defect in these mutants and assess the effect of the mutations on symbiotic nitrogen fixation. Continue studies of the genetics of ammonia assimilation. Use the genetic results to predict the pathways of carbon and nitrogen flow in nodules. Assess the possibility that the malate-aspartate shuttle is an important carrier of energy into bacteroids. We will attempt to locate the critical carrier activities in the bacteroid or peribacteroid membrane. Results of these experiments will provide a text of the nitrogen carrier model.

Progress: 88/01 to 88/12. Work has continued in characterizing the role of glutamine synthetases in Rhizobium meliloti. A mutant unable to express GSII has been shown to have a mutation in the R. meliloti ntrA gene. The ntrA mutant blocks the expression of GSII and a GSII-beta-galactosidase hybrid but does not stop the production of GSI. The GSIII activity

described below is not found in the ntrA mutant. The DNA sequence of the ntrA gene has been determined. It has been suggested that the glutamine synthetase II gene found in Rhizobium and Bradyrhizobium was acquired by the bacteria from their host plants. The DNA sequence of the glutamine synthetase II gene from Rhizobium meliloti has been determine and compared to other glutamine synthetases of this type from plants, animals and bacteria. Although the sequence is about 40% identical to plant and mammalian sequences, the two eukaryotic sequences are 50% identical and therefore are more closely related. We are able to explain these results without requiring a novel eukaryotic to prokaryotic transfer of information. We have purified to apparent homogeneity a unique glutamine systhetase (GSIII) from an R. meliloti mutant that lacks GSI and GSII. This protein has high biosynthetic activity but very low transferase activity. Activity is dependent on divalent cations and ATP and is inhibited by methionine sulfoximine, although at concentrations about 100 fold higher than those that inhibit the other GS proteins.

Publications: 88/01 to 88/12

KAHN, M.L., KRAUS, J. and SHATTERS, R.G. 1988. Bacterial Catabolism of Nitrogen-Containing Compounds in Symbiotic Nitrogen Fixation. In Physiological Limitations and The Genetic Improvement of Symbiotic Nitrogen Fixation.

SHATTERS. R.G. 1988. Glutamine Synthesis in Rhizobium meliloti. Ph.D. Thesis, Washington State University.

KERPPOLA, T.K. and KAHN, M.L. 1988. Symbiotic Phenotypes of Auxotrophic Mutants of Rhizobium meliloti 104A14. J. Gen. Micro. 134:193-199.

KERPPOLA, T.K. and KAHN, M.L. 1988. Genetic Analysis of Carbamoylphosphate Synthesis in Rhizobium meliloti 104A14. J. Gen. Micro. 134:921-929.

67.108 BIOCHEMISTRY OF PLANT CUTICLE

CRISO056197

KOLATTUKUDY P E; Inst of Biological Chemistry; Washington State University, Pullman, WASHINGTON 99164.

Proj. No.: WNPO2001 Project Type: HATCH Agency ID: CSRS Period: 01 DEC 85 to 30 NOV 90

Objectives: The biochemistry of plant cuticle will be investigated to determine the nature of the intermonumer cross links in cutin; determine the structure and mechanism of biosynthesis of suberin; the mechanism of biosynthesis of the novel amino acids, beta-hydroxyphenylalanine and beta-hydroxytyrosine in cutinase; the mechanism of introduction of the novel N-terminus, N-glucuronamide, and other O-glycosidically attached carbohydrates into cutinase; the primary structure of cutinase cDNA and cutinase gene from Colletotrichum capsici; the molecular basis for the drastic differences among Fusarium solani pisi isolates in their ability to produce cutinase. Determine how the unique

cutin monomers induce cutinase synthesis and

define the cutinase promoter. Purify and characterize pectin degrading enzymes involved fungal penetration into plants. Clone cDNA for the pectin degrading enzymes and the genes which code for such enzymes and determine their structure. Determine how the syntheses of the enzymes involved in cuticular/wall penetration are regulated.

Approach: Approach chemical, biochemical and molecular biological approaches will be used to elucidate the structure and biosynthesis of cutin and suberin, the two protective polymers of plants. Recumbant DNA technology will be used to elucidate the mechanism by which pathogenic fungi penetrate their barrier.

Progress: 87/01 to 87/06. Highlights of this project include the determination of the physical and chemical nature of the phytopolymers cutin and suberin and the elucidation of the biochemistry of these protective plant polymers by in vivo and cell-free studies. That these polymers form the primary barrier to microbial infection was proven, and the microbial enzyme responsible for hydrolysis of the polymer (cutinase) was isolated and characterized. Induction of the cutinase enzyme was studied. The cutinase gene from several organisms has been cloned and sequenced, and various fungal phytopathogens have been transformed. Strategies were devised for preventing fungal infection by inhibiting cutinase or altering the responsisble gene and thereby thwarting penetration of the plant surface barrier. Detailed studies on the chemistry and origin of plant waxes were also carried out. A perioxidase involved in suberin formation and a pectin hydrolase involved in fungal infection have also been examined. This work has made a major contribution to understanding the mechanism of fungal penetration of plant cuticle.

Publications: 87/01 to 87/06

KOLATTUKUDY, P.E., ETTINGER, W.F. and SEBASTIAN, J. 1987. Culticular lipids in plant-microbe interactions. In Stumpf, P.K., Mudd, J.B. and Nes, W.D. (eds) "The Metabolism, Structure, and Function of Plant Lipids." Plenum Press, New York.

KOLATTUKUDY, P.E., BOHNET, S., ROBERTS, E. and ROGERS, L. 1987. Peroxisomes in sebaceous glands: biosynthetic role and hormonal regulation. In Fahimi, H.D. and Sies, H. (eds) "Peroxisomes in Biology and Medicine." Springer-Verlag, Berlin.
KOLATTUKUDY, P.E., BOHNET, S. and ROGERS, L.

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67.109* CRISO141948 MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONADS

WILLIS D K; Agricultural Research Service, Madison, **WISCONSIN** 53702.

Proj. No.: 3655-24000-003-00D

Project Type: INHOUSE Agency ID: ARS Period: 30 MAR 87 to 29 MAR 92 **Objectives:** Molecular genetic analysis of the genes and gene products involved in pathogenicity, toxin production, and toxin resistance in two phytopatho- genic pseudomonads, P. s. syringae and P. s. tabaci.

Approach: Phenotypic variants in pathogenicity, tabtoxin production, or tabtoxinine- B-lactam resistance will be generated by transposon mutagenesis. The wild- type genes involved in these processes are isolated from cosmid genomic libraries by either direct restoration of phenotype, or by utilizing the transposon as a molecular hook. The genes will be mapped, sequenced, and the gene products identified. Cloned genes will also be utilized as molecular probes for the investigation of genetic evolution, taxonomic rela- tionships, and disease diagnostic probes. MADISON, WI, PDRR; RM. XX: BL-X; DR. D. K. WILLIS (equipment pending).

Progress: 88/01 to 88/12. The continuing analysis of a mutant strain of Pseudomonas syringae pv. syringae has indicated that syringomycin and/or protease production is involved in lesion formation by this pathogen on bean. This is the first indication of possible biological mechanism for the disease symptoms caused by leaf-spot bacterial pathogens. An recA deficient derivative of a pathogenic P. s. pv. syringae isolate was characterized. This mutation does not significantly alter the pathogenic response. This genetic background is being used in the above analysis of pathogenicity of a recombination deficient background into this system for the study of the molecular genetics of pathogenicity. A DNA region required for tabtoxin production and resistance by both P. s. pv. tabaci (wildfire of tobacco) and P. syringae isolate BR2 (wildfire bean) was recently identified and cloned. This has resulted in the isolation of dianostic DNA probes that distinguish tabtoxin producing strains from nonproducing strains. It has also been established that P. s. angulata can be derived from P.s. pv. tabaci by deletion of the cloned region which suggests that the two major bacterial diseases of tobacco (wildfire and angular leafspot) are caused by derivatives of the same organism.

Publications: 88/01 to 88/12

BARTA, T.M., KINSCHERF, T.G., CDLEMAN, R.H. and WILLIS, D.K. 1988. Molecular analysis of a common DNA swquence involved in tabtoxin production by Pseudomonas syringae. Accepted by Phytopathology. Accepted Nov. 3, 1988.

HRABAK, E.M. and WILLIS, D.K. 1988. Effect of a recA mutation...P.s.syringae pv.

syringae on its growth and pathogencity, p. 176. In: N.T. Keen, T. Kosuge, and L.L Walling (eds.) Physio and Biochem of Plant-Micro Inter. Amer. Soc., MD.

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WILLIS, D.K., KINSCHERF, T.G., BARTA, T.M. and CDLEMAN, R.H. 1988. Isolation ...of Tn5 insertions...resistance in P.s. pp. 261-262

In: D.P. Verma, and R. Palacios (ed.).
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HRABAK, E.M., and WILLIS, D.K. 1988. Effect of a recA mutation in P.s. pv.

syringae on its growth and pathogenicity, p. 176. In: N. Keen, T. Kosuge and L.

67.110 CRISO130295 REGULATION OF PLANT GENE EXPRESSION BY DNA METHYLATION

AMASIND R M; Biochemistry; University of Wisconsin, Madison, **WISCONSIN** 53706.

Proj. No.: WISO3081 Project Type: STATE Agency ID: SAES Period: 15 JUN 86 to 30 JUN 92

Objectives: To determine the DNA sequence elements of the T-RNA oncogene encoding an isopentenyl transferase essential for gene expression, to map the regions of this gene in which DNA methylation inhibits transcription, and to investigate the role of DNA methylation in plant chromatin structure.

Approach: Dur approach includes deletion and linker-scanning mapping of the promoter of the isopentynyl transferase gene fused to the chloramphenicol acetyl transferase reporter gene, mapping the sites of demethylation in revertant clones that have re-initiated T-DNA expression either spontaneously (at a frequency of 10 7) or in response to 5-azacytidine treatment by use of methylation-sensitive restriction enzymes and genomic sequencing, methylating this T-DNA in vitro at certain sites and monitoring the effect of methylation on expression after introducing this modified gene into cells by electroporation, and examining the DNAase I sensitivity of our methylation mapped constructs to assess the effect of methylation on chromatin structure.

Progress: 88/01 to 88/12. We have constructed a delection map of the 5' region of theipt gene from the T-DNA of the A. tumefaciens Ti plasmid. This analysis has revealed previously undetected upstream regulatory sequences necessary for maximal levels of expression of this gene in tobacco cells. We have optimized conditions for the demethylation of DNA in the plant genome by 5-azacytidine. These studies have revealed that plant genomes can be substantially demethylated at cytosine residues by this drug, that demethylation results in the rapid induction of expression of silent T-DNA genes and that plant genomes recover normal levels of cytosine methylation after removal of the 5-azacytidine. We have demonstrated that the DNAse I-sensitive fraction of the plant genome is depleted in 5-methycytosine. The DNAse I sensitive fraction

is thought to represent active plant genes. These results therefore indicate that DNA methylation may play a role in the organization of plant genomes into active and inactive domains.

Publications: 88/01 to 88/12 REID, R.A., JOHN, M.C. and AMASINO, R.M. 1988. Deoxyribonuclease I sensitivity of the T-DNA ipt gene is associated with gene expression. Biochemistry 27:5748-5754.

KLAAS, M., JOHN, M.C., CROWELL, D.N. and AMASINO, R.M. 1988. Rapid induction of genomic demethylation and T-DNA Gene Expression in Plant Cells by 5-Azacytosine derivatives. Plant Mol. Biol., accepted.

KLAAS, M. and AMASINO, R.M. 1988. DNA methylation is reduced in DNase I-sensitive regions of plant chromatin. Plant Physiology, submitted.

67.111 CRTS0098948 THE THYLAKOID ENERGY TRANSDUCING ATPASE COMPLEX

SELMAN B R; Biochemistry; University of Wisconsin, Madison, WISCONSIN 53706. Proj. No.: WISO2419 Project Type: STATE Agency ID: SAES Period: O1 JUL 86 to 30 SEP 91

Objectives: We have been studying the chloroplast thylakoid energy transducing H -dependent ATP synthase (CF(0)-CF(1)) complex both in higher vascular plants (spinach) and in unicellular, green algae (Chlamydomonas reinhardi and Dunaliella). We have made the following progress: A) using substrate photoaffinity adenine nucleotide analogs modified both in the adenine base and the ribose ring, we have begun to map the catalytic site(s) of the ATP synthase both in situ and in vitro. B) Using covalent chemical modifiers specific for certain amino acids, we have begun an investigation of the subunit bold center dot subunit interactions of the CF(1). C) We have finally been successful in partially purifying the active CF(0)-CF(1) complex from the thylakoid membranes of the alga C. reinhardi. D) We have been successful in raising monospecific, polyclonal antisera (in rabbits) directed against the algal CF(0) polypeptides.

Approach: These last two accomplishments represent extremely important advances for us for our studies on the biogenesis of the ATP synthase complex and our analysis of ATP synthase defective mutant algal strains.

Progress: 88/01 to 88/12. Our research efforts over the past year have focused on both the enzymology and biogenesis of the plant chloroplast energy transducing ATP synthase (CF(0) CF(1) complex. Enzymology: We have been studying both the in vivo and in vitro regulation of the catalytic activity of the complex. A-i) Light-activation of the thylakoid membrane-bound ATP synthase is associated with the reduction of a disulfide bridge on the gamma-subunit of the CF(1) sector of the complex. Inactivation in the dark, after the protonmotive force has decayed, is accompanied by the re-oxidation of the vicinal dithiols. The chemical nature of the oxidant remains to

be determined; however, we have developed a purification protocol for it and have measured the redox difference spectrum (epsilon (max) 240 nm). A-ii) Release of the CF(1) sector from thylakoid membranes by chloroform extraction yields a heterogeneous population of CF(1). This population can be fractionated by HPLC anion exchange chromatography. Those isozymes lacking the epsilon-subunit are active as an ATPase. An analysis of the causes for the distribution of isozymes has shown that the presence of dithiothreitol leads to the reversible dissociation of the epsilon-subunit from holoenzyme or CF(1) lacking the delta-subunit. Biogenesis: B-i) The cDNA sequence and thereby the deduced amino acid sequence of the complete C. reinhardtii CF(1) gamma-subunit has been determined.

Publications: 88/01 to 88/12

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- SELMAN-REIMER, S. and SELMAN, B.R. 1988. The partial purification of a factor from Dunaliella salina that causes the rapid in situ inactivation of light-activated chloroplast coupling factor 1 (CF(1)), FEBS Lett. 230:21-24.
- YU, L.M., MERCHANT, S., THEG, S.M. and SELMAN, B.R. 1988. Isolation of a cDNA clone for the gamma subunit of the chloroplast ATP synthase of Chlamydomonas reinhardtii: Import and cleavage of the precursor protein. Proc. Natl. Acad. Sci.

67.112* CRIS0132673 MOLECULAR TAGGING OF THE RP LF GENE IN MAIZE

ELLINGBOE A H; QIN M; ROBERTSON W; Plant Pathology; University of Wisconsin, Madison, WISCONSIN 53706.

Proj. No.: WISO3172 Project Type: CRGO Agency ID: CRGO Period: 15 JUL 87 to 31 DEC 89

Objectives: PROJ. 8700291. Clone the DNA segments that contain Mu-1. Determine the complementation between descendants of the 38 independently induced mutants.

Approach: A library of mutant D-4 has been prepared in lambda EMBL3. We are now screening for plaques containing Mu-1. Plaques that contain an Hind III fragment of approximately 7.0 Kb will be used in experiments to determine which contains flanking sequences that segregate with the Rplf gene. Mutants will be intercrossed to determine which mutants will compliment to restore the resistance phenotype.

Progress: 88/01 to 88/12. We have continued to try to determine which of the many copies of Mu is in he Rpl locus. A library of one mutant, D-4, was prepared in lambda EMBL3. Out of more than 2.2x10 plaques screened, 78 positives were identified using pMuED2 as probe. DNA prepared from 42 clones were digested with one or more enzymes, electrophoresed, and probed with pMuED2. All hybridized with pMuED2 but only 20 hybridized with the internal fragment

of Mu1. The blots were also probed with the unique fragments of Mus3, 4, 5, 6, 7, and 8(obtained from V. Chandler). Four clones hybridized with Mu5 and 3 hybridized with Mu8. Two restriction fragment length polymorphisms (RFLPs) (285 and 422 from NPI) show polymorphisms in the Rp1 region on chromosome 10 to 5 corn lines isogenic except for 5 different alleles at the Rp1 locus. Two probes, prepared by primer extension away from the ends of Mu of two clones 6-1-1 and 6-22, also showed polymorphisms when the DNA of 5 isogenic 1nes was cut with either of 2 enzymes. No polymorphisms among the isogenic lines were observed with probes prepared with the other lambda clones. Spm has now been transferred into plants with Rp1D, Rp1e or Rp1k by crossing following a procedure used for Mul. None has yet been screened for mutations at the Rp1 locus.

Publications: 88/01 to 88/12

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BENNETZEN, J.L., QIN, M.-M., INGELS, S. and ELLINGBOE, A.H. 1988. Allele specific and mutator-associated instability at the Rp1 disease resistance locus of maize. Nature 332:369-370.

67.113 CRISO027435 HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS

SEQUEIRA L; LEONG S; Plant Pathology; University of Wisconsin, Madison, **WISCONSIN** 53706.

Proj. No.: WISO1474 Project Type: STATE Agency ID: SAES Period: O1 JUL 84 to 30 JUN 92

Objectives: The main objective is to examine the structural and physiological factors involved in the recognition response of plants to invasion by bacteria. We want to explore the strong correlation that exists between incomplete ("rough") lipopolysaccharide (LPS) and ability of certain strains of Pseudomonas solanacearum (e.g. B1) to attach to plant cells and to induce a hypersensitive response (HR). This is possible now because we have constructed a suicide plasmid that carries the transposable element, Tn 5, that causes mutations by inserting at random in the P. solanacearum genome.

Approach: To obtain a wide variety of avirulent mutants of P. solanacearum by transposon mutagenesis, to analyze mutants for their capacity to induce the HR and for changes in other factors thought to be associated with virulence, to clone DNA from selected mutants in E. coli and to use portions of the DNA flanking the transposon to identify clones carrying similar sequences in a gene bank of the wild, parental strain, to reintroduce the clones carrying the flanking sequences into P. solanacearum mutants and to test the transconjugants for restoration of the wild type phenotype. Once the limits of the complementing activity for HR induction or for

virulence functions are determined, the products of the genes contained in the clone fragment will be isolated.

Progress: 88/01 to 88/12. Progress Report. The general objective of this project is to define the genetic system that controls expression of the hypersensitive response (HR) elicited by Pseudomonas solanacearum in plant hosts and non-hosts. Previously, we had obtained a series of genetically-defined. transposon generated mutants from both HR HR parental strains. Because many of the mutants were avirulent, HR , and produced no extracellular polysaccharide (EPS) in culture, efforts this past year centered on attempts to determine the relationship of altered colony morphology to virulence and HR induction. By means of Tn5 mutagenesis, however, we have isolated a class of mutants that have an afluidal colony morphology but retain the ability to cause severe wilting and death of tobacco plants. One such mutant, KD700, was studied in detail. By marker exchange mutagenesis, the altered colony morphology was shown to be the result of a single Tn5 insertion in a 14.3 kb EcoR1 fragment. This defect could be corrected by introducing a homologous clone from a cosmid library of the wild-type, parental strain K60. The Tn5-containing fragment was introduced into other P. solanacearum wild-type strains by marker exchange, and these altered strains had the same phenotype of KD700. Galactosamine, a major constituent of EPS of all wild-type strains of P. solanacearum, was not detected by gas-chromatographic analysis of liquid cultures of KD700 or of vascular fluids from plants infected by KD700.

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Publications: 88/01 to 88/12

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CM 70 RESEARCH EQUIPMENT AND TECHNOLOGY

70.001* CRISO089967
CELLULAR AND MOLECULAR GENETICS FOR CROP
IMPROVEMENT

GURLEY W B; INGRAM L O; INGRAM L O; Microbiology & Cell Science; University of Florida, Gainesville, **FLORIDA** 32611. Proj. No.: FLA-MCS-O2317 Project Type: HATCH Agency ID: CSRS Period: O1 OCT 82 to 30 SEP 88

Objectives: Identification and characterization of agriculturally important genetic systems. Regulation of gene expression and the delivery of genetic material to higher plants and associative microorganisms.

Approach: Agriculturally important genes in the areas of hydrogen uptake in nodulated legumes, alcohol tolerance in microorganisms, and UV radiation damage will be identified and characterized using a variety of genetic and biochemicals methods. The regulation of the transcriptional aspects of gene expression in higher plants will be studied using the T-DNA of Agrobacterium tumefaciens Ti plasmid as model plant genes. The Ti plasmid will also be utilized in the development of a vector for the introduction of genetic material into plant cells.

Progress: 87/10 to 88/09. We have cloned a positive element that resides upstream of the Adh-1 promoter of maize. This TATA-distal promoter element (located from position -410 to -140) contributes 50% of the transcriptional activity of this gene. We are currently analyzing the effects of distance, position, and polarity of orientation on transcriptional activity. We have also constructed a series of vectors that will test the effect of substitution of the 780 gene activator (T-right pTi 15955) on core the promoter of Adh-1. The promoter constructs will be expressed transgenetically in sunflower tumors. S1 nuclease hybrid protection mapping of poly(A) RNA will be used for analysis of mutant transcriptional activity.

Publications: 87/10 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

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KEYWORD/TITLE INDEX

A-BETA-ALLELES

ISOLATING AND CHARACTERIZING A-BETA MATING-TYPE ALLELES OF SCHIZOPHYLLUM COMMUNE.. 66.013, 67.102*

ABIES-PROCERA

GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014

ABSCISIC-ACID

ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS OFFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND OEVELOPMENT. 67.015

MECHANISMS DIRECTING HORMONAL AND OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035

REGULATION OF GENE EXPRESSION BY ABSCISIC ACIO OURING OROUGHT STRESS. 67.016

ABSCISTIC-ACID

REGULATION OF PLANT GENE EXPRESSION BY CELL TURGOR OR ABSCISIC ACIO. 67.093

ACALYPTRATA

GENETICS AND BREEDING OF COOL SEASON CROPS.
12.011

ACCESSIONS

RFLP METHOOOLOGY FOR RELATEONESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

ACCLIMATIZATION

ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

ACETYLENE

MOOULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021

ACID-PHOSPHATASE

ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELOIOOGYNE INCOGNITA. 67.053 MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO. 65.001, 67.012*

ACROTRISOMICS

ANEUPLOIO ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007

ACTIN

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

ACTINORHIZAE

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. **06.018**, **12.048***, **14.079***

ACTIVATION

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008

ACTIVE-SITES

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. 67.044

ADENOSINE-TRIPHOSPHATASE

THE THYLAKOIO ENERGY TRANSOUCING ATPASE COMPLEX. 67.111

ADP-GLUCOSE-PYROPHOSPHORYLASE.

OISSOCIATION MUTAGENESIS OF THE SHRUNKEN-2 LOCUS OF MAIZE. 14.012

AEGILOPS

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*

AFFINITY-CHROMATOGRAPHY

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069

AGARICUS-BISPORUS

GERMPLASM ENHANCEMENT AND CULTURE OF EOIBLE MUSHROOMS. 12.038

AGMENELLUM-QUADRUPLICATUM

THE PHYCOCYANIN GENES OF AGMENELLUM QUADRUPLICATUM. 67.088

AGROBACTERIUM

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*

OEFINING ANO MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*

OEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS. O6.008

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*

MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO. **65.001**, **67.012***

ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055

TOWAROS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

AGROBACTERIUM-TUMEFACIENS

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*, 70.001*

GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033
GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF
HOST-PATHOGEN INTERACTION. 10.008, 66.012*
MOLECULAR GENETIC APPROACHES FOR GERMPLASM

DEVELOPMENT OF FRUIT AND NUT CROPS. 10.001

AGROCIN

BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*

AGRONOMY

CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014

GENETIC MECHANISMS IN CORN. 14.044

GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017. 23.010*

PHYSIOLOGICAL. BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035

PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.016

AGROTIS-IPSILON

METHOOS OF CONTROLLING CORN INSECTS. 14.024

AIR-TEMPERATURE

SOYBEAN BROWN STEM ROT. 23.008

ALCOHOL-DEHYDROGENASE

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008

DEVELOPMENTAL GENETICS USING THE ALCHOL OEHYOROGENASE GENE-SYSTEM IN MAIZE. 14.004 GENETIC MANIPULATION OF LACTIC OEHYOROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019

ALFALFA

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

CYTOGENETIC MANIPULATIONS FOR ALFALFA

IMPROVEMENT. 20.002
INHERITANCE OF MITOCHONORIAL ONA IN SOMACLONAL VARIANTS. 20.003

ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015

MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*

SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

ALGAE

THE THYLAKOIO ENERGY TRANSOUCING ATPASE COMPLEX. 67.111

ALIEN-CHROMOSOMES

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*

ALKALOIDS

CELL SPECIFIC GENE EXPRESSION OURING LATICIFER DIFFERENTIATION IN POPPY. 67.096 ALLELES

ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE FUNCTION AND REGULATION. 14.054

ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058, 14.063

MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA. 67.069

MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. **67.067**

MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021

RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN. 23.017

TISSUE-SPECIFIC GENE REGULATION IN MAIZE. 14.050

ALLELISM

GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

ALLELOPATHY

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008. 18.011*

ALLOZYME

DEVELOPMENT OF NEW AND IMPROVEO CROPS FOR WATER CONSERVATION IN ARIO LANOS. 25.004

ALLOZYMES

18.003. 67.006*

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE

OF HYBRIDIZING POPULATIONS. 14.051
GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*, 21.005*, 23.040*

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009

GENETICS OF BIOTYPES IN THE HESSIAN FLY (MAYETIOLA OESTRUCTOR). 17.014

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. 06.013

POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS. 21.004 RFLP METHOOOLOGY FOR RELATEONESS AMONG SMALL

GRAINCEREAL ACCESSIONS. 18.002

ALNUS-GLUTINOSA

ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. 67.092

ALPHA-AMYLASES

CELLULAR REGULATION OF THE EXPRESSION OF DORMANT GENES IN CEREAL GRAINS, 62,002 ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017

THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001

ALSTROEMERIA

BARLEY GENETICS AND PLANT CYTOGENETICS. 18.008, 20.004*, 27.001*, 67.022* **AMARANTHUS**

PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE. ANO DISTRIBUTION. 25.001

AMINO-ACID-COMPOSITION

CONTROL OF THE BIOSYNTHESIS OF PROTEINS & AMINO AC IOS IN LEGUME SEEOS. 23.046

AMINO-ACID-METABOLISM

AMINO ACIO METABOLISM ANO THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*

AMINO-ACID-SEQUENCE

ANALYSIS OF SEED GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033 CELLULAR REGULATION OF THE EXPRESSION OF DORMANT GENES IN CEREAL GRAINS. 62.002

EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*

EVOLUTION OF POLYPLOID WHEATS VIA AMINO ACID SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*

GENETIC MANIPULATION OF LACTIC DEHYOROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019

ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELOIDOGYNE INCOGNITA. 67.053

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED

6 1

ATRAZINE TOLERANCE IN CORN. 14.069
REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNOS IN HIGHER PLANTS.
67.045

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE ANO METABOLISM OF ITS PRODUCTS. 67.044

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046
THE MOLECULAR BIOLOGY OF THE RICE
ALPHA-AMYLASE GENES. 16.001

THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION. 18.036, 63.007*

AMINO-ACID-SYNTHESIS

BIOCHEMICAL ANO OEVELOPMENTAL GENETICS OF HIGHER PLANTS. 14.038, 18.020 CONTROL OF THE BIOSYNTHESIS OF PROTEINS &

AMINO AC IOS IN LEGUME SEEOS. 23.046

AMINO-ACIDS

AMINO ACIO METABOLISM ANO THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*

BIOCHEMISTRY OF PLANT CUTICLE. **67.108**CONTROL OF THE BIOSYNTHESIS OF PROTEINS &
AMINO AC IOS IN LEGUME SEEOS. **23.046**

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING ANO ELECTROPHORETIC STUDIES. 17.004, 18.005*

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015
MAPPING, TRANSFER, RECOMBINATION AND
EXPRESSION OF ORGANELLE AND NUCLEAR GENES.
16.004, 17.020*, 23.031*, 67.056*

AMPHIDIPLOIDS

GENOME EVOLUTION IN BRASSICA. 12.012

AMYLASES
MECHANISMS DIRECTING HORMONAL AND

OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026
ORGANIZATION AND EXPRESSION OF A-AMYLASE

ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. **62.001** ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. **18.017**

AMYLOPLASTS

BIOCHEMICAL ANO OEVELOPMENTAL GENETICS OF HIGHER PLANTS. 14.038, 18.020 MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. 67.089

ANABAENA

STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED OURING NITROGEN FIXATION. 67.084

ANAEROBIC-CONDITIONS

GENETIC MANIPULATION OF LACTIC OEHYDROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019

ANALYSIS

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

ANALYTICAL-CHEMISTRY

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACID SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*

AND

CELLULAR ANO MOLECULAR BIOLOGY OF SUGARCANE. 27.003

ANEUPLOIDS

ANEUPLOIO ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007

BARLEY GENETICS AND PLANT CYTOGENETICS. 18.008, 20.004*, 27.001*, 67.022*

CHROMOSOMAL LOCALIZATION AND INTRACHROMOSOMAL MAPPING OF WHEAT RFLP LOCI. 17.032

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN

TRITICEAE SPECIES. 17.030, 18.032*
CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT

HROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIDIZE TO UNIQUE-SEQUENCE-ONA CLONES. 17.031

CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015

GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOID WHEAT AND ITS RELATIVES. 67.097

GENETIC ANO CYTOGENETIC ANALYSIS OF AGRONOMIC ANO QUALITY CHARACTERISTICS OF OURUM ANO COMMON WHEAT. 17.025

GENETIC MECHANISMS IN CORN. 14.044

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015
GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON
GENE MAPPING 23.016

GENE MAPPING. 23.016
GENETICS, TISSUE CULTURE, AND MOLECULAR
BIOLOGY OF THE SOYBEAN. 23.018

GENOME EVOLUTION IN BRASSICA. 12.012

GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001

PRESERVATION ANO UTILIZATION OF GERMPLASM IN COTTON. 21.016

STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE & THEIR USE AS RESEARCH TOOLS. 14.018

ANIMAL-GENETICS

CONSULTATION ANO RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS. 14.032, 23.024*, 30.001*, 31.002*

ANNUAL-LEGUMES

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

ANTAGONISTS

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEOLING OIHYOROFOLATE REOUCTASE. 23.053

ANTHOCYANINS

GENETIC MECHANISMS IN CORN. 14.044
ORGANIZATION OF THE R CHROMOSOME REGION IN
MAIZE. 14.077

TOWARDS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

ANTHRACNOSE

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

ANTIBODIES

CHARACTERIZATION OF NITROGEN FIXATION GENES ANO THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

FUNCTION OF PLANT RNA-DEPENDENT RNA POLYMERASES. 12.036

GENETIC MANIPULATION OF LACTIC DEHYDROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035

THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. 67.054

ANTIBODY-PRODUCTION

EXPRESSION OF MAIZE MITOCHONDRIAL GENOME. 14.002

ANTICUTINASE

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

ANTIGENS

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*

ANTIOXIDANTS

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

ANTIPENETRANTS

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

ANTISERA

CELL SPECIFIC GENE EXPRESSION OURING LATICIFER DIFFERENTIATION IN POPPY. 67.096

ARABIDOPSIS

TUBULIN GENES OF PLANTS. 67.060

A STUDY OF GENETIC DIVERSITY IN PEANUTS USING CHLOROPLAST AND RRNA MOLECULAR MARKERS. 24.002

ARBUSCULAR

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA & SMALL GRAINS COLLECTIO. 17.008, 18.011*

AROMATIC-COMPOUNDS

REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNDS IN HIGHER PLANTS. 67 045

ASPARTATE

AMINO ACIO METABOLISM ANO THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014. 67.107*

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

ASPARTOKINASE

MAPPING, TRANSFER, RECOMBINATION AND XPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

ASPERGILLUS-FLAVUS

GENETICS OF ASPERGILLUS FLAVUS. 67.036

SYSTEMS FOR THE MOLECULAR ANALYSIS OF OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

ATRAZINE

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069

THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN ANO COTTON. 14.068, 21.007*

AUXIN

THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIDASE. 26.005

AUXOTROPHY

GENETICS OF ASPERGILLUS FLAVUS. 67.036

ΔVFNΔ

18.003, 67.006*

AVOCADOS

CELLULASE GENE EXPRESSION OURING FRUIT OEVELOPMENT. 67.004

MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017

AXENIC-CULTURE

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

AZOSPIRILLUM

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103

AZOTOBACTER-VINELANDII

CHARACTERIZATION OF NITROGEN FIXATION GENES ANO THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057

GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANOII. 23.048, 23.049, 66.006*, 66.007*

BACILLUS-THURINGIENSIS

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. **06.015**

METHODS OF CONTROLLING CORN INSECTS. 14.024

BACKCROSSING CHROMOSOMAL MAPPING OF GENES CONTROLLING

TISSUE CULTURE RESPONSE IN WHEAT. 17.010 CYTOPLASMIC FACTORS OF THE POTATO. 11.012 OEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELD AND GENE EXPRESSION. 67.018

GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011

BACTERIA

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010

BACTERIAL GENES CODING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098 BIOLOGICAL CONTROL OF PLANT PATHOGENIC

BACTERIA. 10.002, 12.018* CELLULAR AND MOLECULAR GENETICS FOR CROP

IMPROVEMENT. 18.013, 26.002*, 63.002, 66.002*, 67.026*, 67.035, 70.001*

CHARACTERIZATION OF NITROGEN FIXATION GENES

AND THEIR PRODUCTS FROM AZOTOBACTER VINELANDII. 23.057 DEVELOPMENT OF A ONA HYBRIDIZATION ASSAY FOR

DETECTION OF THE RING ROT PATHOGEN. 11.007 EXPRESSION OF MAIZE MITOCHONDRIAL GENOME. 14.002

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GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVEO AGRONOMIC PROPERTIES. 23.039

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 12.010, 17.023*, 18.029*

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE AND F000 VALUE. 12.021

GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANDII. 23.048, 23.049, 66.006*, 66.007*

GLYPHOSATE DEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*
HOST-PATHOGEN RECOGNITION AND DISEASE

RESISTANCE IN PLANTS. 67.113

MODULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006 MOLECULAR GENETICS OF NITROGEN METABOLISM IN

RHIZOBIA. 23.022 PLASMIOS IN PLANT PATHOGENIC BACTERIA.

18.012, 26.001* THE PHYTOPATHOGENIC BACTERIA. 67.052

BACTERIAL-BLIGHT

MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

BACTERIAL-CANKER

BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*

BACTERIAL-DISEASES-(PLANTS)

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010 BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*

BIOLOGY, EPIOEMIOLOGY, GENETICS. AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

CELLULAR AND MOLECULAR GENETICS FOR CROP

IMPROVEMENT. 67.035

- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 12.010, 17.023*, 18.029*
- GENETIC MOSAICS ANO THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS ANO OISEASES. **06.003**
- HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002
- MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*
- MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034
- MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*
- MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*
- PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

BACTERIAL-GENETICS

- AMINO ACIO METABOLISM ANO THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*
- BACTERIAL GENES CODING FOR PLANT RIBULOSE
 BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098
 CELLULAR AND MOLECULAR GENETICS FOR CROP
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.035
- CHARACTERIZATION OF NITROGEN FIXATION GENES AND THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057
- EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010
- GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*
- GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*
- GLYPHOSATE DEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*
- HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002
- HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113
- MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034
- MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*
- MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022
- PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*
- STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED OURING NITROGEN FIXATION. 67.084
- THE REPLICATION OF CHLOROPLAST DNA IN CHLAMYDOMONAS REINHARDII. 67.057

BACTERIAL-PHYSIOLOGY

- AMINO ACID METABOLISM AND THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*
- MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022

BACTERIAL-RING-ROT

RECOMBINANT ONA APPROACH TO DETECTION AND STRAIN DIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009

BACTERIAL-SPECK

BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*

BACTERIAL,

- BASIC AND APPLIED MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN. 11.008
- THE PHYTOPATHOGENIC BACTERIA. 67.052 BACTERIOLOGY
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.035
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010

BACTERIOPHAGES

- CELL SPECIFIC GENE EXPRESSION OURING LATICIFER DIFFERENTIATION IN POPPY. 67.096
- GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*
- ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*
- MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*
- ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017
- PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO OISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

BACULO-VIRUSES

- IMPROVING THE EFFICACY OF BACULOVIRUS
 PESTICIDES BY RECOMBINANT ONA TECHNOLOGY.
 65.002, 67.031*
- ORGANIZATION AND EXPRESSION OF A BACULOVIRUS DNA GENOME. **65.004**, **67.040***

BARLEY

- ANEUPLOID ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007
- BARLEY BREEDING AND GENETICS. 18.027
- BARLEY GENETICS AND PLANT CYTOGENETICS. 18.008, 20.004*, 27.001*, 67.022*
- BIOCHEMICAL AND DEVELOPMENTAL GENETICS OF HIGHER PLANTS. 14.038, 18.020
- CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002
- GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 17.023*, 18.029*, 67.104
- GENETIC MANIPULATION OF LACTIC DEHYOROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019
- GENETICS OF NITRATE REDUCTION IN BARLEY. 67.105
- GERMPLASM ENHANCEMENT AND IMPROVEO BREEDING METHOO S IN BARLEY. 18.001
- MECHANISMS DIRECTING HORMONAL AND OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026
- MOLECULAR AND GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034
- ORGANIZATION AND CONTROL OF THE HYDROLASE GENES IN BARLEY. 18.018
- ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. 62.001
- ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017
- RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*
- RFLP METHODOLOGY FOR RELATEONESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002
- STRUCTURE AND EXPRESSION OF PLANT GENES

ENCODING CALMODULIN. 18.014

USE OF SINGLE COPY CLONED DNA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028

BASIDIOMYCETE

ISOLATING AND CHARACTERIZING A-BETA MATING-TYPE ALLELES OF SCHIZOPHYLLUM COMMUNE.. 66.013, 67.102*

BASIDIOMYCETES

CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*

BEANS

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELD, PEST RESISTANCE AND FOOD VALUE. 12.021

HYBRID VARIEGATION IN PHASEOLUS VULGARIS. 23.038

BEAUVERIA-BASSIANA

METHODS OF CONTROLLING CORN INSECTS. 14.024

THE THYLAKOID ENERGY TRANSDUCING ATPASE COMPLEX. **67.111**

BIOASSAYS

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.010

DEVELOPMENT OF A DNA HYBRIDIZATION ASSAY FOR DETECTION OF THE RING ROT PATHOGEN. 11.007 SOYBEAN BROWN STEM ROT. 23.008

BIOCHEMICAL-GENETICS

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 18.013, 23.006*, 24.001*, 26.002*, 63.002, 66.002*, 67.026*, 67.028, 70.001* CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF

DS ELEMENT. 14.053

CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. **67.014**

EXPRESSION OF EMBRYO-SPECIFIC GENES DURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*

GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027

GENETICS AND CYTOLOGY OF MAIZE. 14.022 GENETICS OF NITROGEN FIXATION IN AZOTOBACTER

VINELANDII. 23.048, 66.006* ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM

ALFALFA. 20.015 ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017

REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSION. 23.001

STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE. 23.035

THE REPLICATION OF CHLOROPLAST DNA IN CHLAMYDOMONAS REINHARDII. 67.057

BIOCHEMICAL-MECHANISMS

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIDS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

BIOCHEMISTRY

BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033 CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE. 27.003

CHARACTERIZATION OF NITROGEN FIXATION GENES AND THEIR PRODUCTS FROM AZOTOBACTER VINELANDII. 23.057

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046

BIOCHEMISTRY-OF-OIL-BIOSYNTHES

GENETIC ENGINEERING OF OILSEED SPECIES TO IMPROVE OIL QUALITY. 23.020, 25.003*

BIOLOGICAL-CONTROL-(DISEASES)

BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*

ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018. 12.048*, 14.079*

PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAD DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

BIOLOGICAL-CONTROL-(INSECTS)

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. 06.015

GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*, 21.005*, 23.040*

IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIDES BY RECOMBINANT DNA TECHNOLOGY. 65.002, 67.031*
METHODS OF CONTROLLING CORN INSECTS. 14.024

BIOLOGICAL-CONTROL-(WEEDS)

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018, 07.001, 12.048*, 14.079*, 17.023*, 18.029*

0 1

BIOLOGY

CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE. 27.003

BIOLUMINESCENT

THE MOLECULAR BASIS OF BLACK ROT OF CRUCIFERS. 12.001

BIOMASS

CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*

MOLECULAR MANIPULATION OF GENES. 28.002

BIOPULPING

MOLECULAR BIOLOGY OF CELLULOSE AND LIGNIN DEGRADATION BY PHANEROCHAETE CHRYSOSPORIUM. 06.017

BIOREGULATION

CELLULAR REGULATION OF THE EXPRESSION OF DORMANT GENES IN CEREAL GRAINS. 62.002

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIDS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

CELLULAR METABOLISM IN PLANTS. 67.049 CEREAL GRAIN IMPROVEMENT BY RECOMBINANT DNA METHODS. 17.007

CONTROL OF THE BIOSYNTHESIS OF PROTEINS & AMINO AC IDS IN LEGUME SEEDS. 23.046

ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIDE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027
ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS

DEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACID. 14.001

BIOTECHNOLOGY

13.002

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*

BIOTYPES

GENETICS OF BIOTYPES IN THE HESSIAN FLY (MAYETIOLA DESTRUCTOR). 17.014

BLACK-ROT

THE MOLECULAR BASIS OF BLACK ROT OF CRUCIFERS. 12.001

CLONING CULTIVAR SPECIFICITY GENES FROM MAGNAPORTHE GRISEA. 16.010

BLUE-GRAMA

BARLEY GENETICS AND PLANT CYTOGENETICS. 18.008, 20.004*, 27.001*, 67.022*

BRACKISH-WATER

GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001

BRADYRHIZOBIUM-JAPONICUM

MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022

BRANCHES

GENETIC CONTROL OF PLANT MORPHOGENESIS:
STUDIES ON TWO HOMEOTIC MUTANTS DF MAIZE.
14.065

BRASSICA

EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*

GENOME EVOLUTION IN BRASSICA. 12.012, 12.013
MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN
ANTHERS OF BRASSICA. 67.069

MOLECULAR ANALYSIS OF THE S LOCUS OF BRASSICA. 67.068

MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067

BRASSICA.

MOLECULAR MARKERS FOR BRASSICA CAMPESTRIS CHROMOSOMES. 12.026

BREAKAGE

TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*

BREEDER-SEED

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060

BREEDING

A STUDY OF GENETIC DIVERSITY IN PEANUTS USING CHLORDPLAST AND RRNA MOLECULAR MARKERS. 24.002

DEVELOPMENT OF A MOLECULAR CYTOGENETIC MAP OF COTTON BY IN SITU HYBRIDIZATION. 21.012

BREEDING-LINES

ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
CONSTRUCTION AND CHARACTERIZATION OF SOMATIC
HYBRIDS AND CYBRIDS OF TOMATO. 12.030

CRUCIFER DISEASES. 12.047

DEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELD AND GENE EXPRESSION. **67.018**

DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.056

GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF DURUM AND COMMON WHEAT. 17.025

GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011

GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008

MAIZE GRAIN PROTEIN COMPOSITION AND DISTRIBUTION AS RELATED TO HARDNESS, HANDLING AND PROCESSING. 14.016

MAPPING THE SYMBIOSIS GENES OF PEA. 67.077
MOLECULAR GENETIC CHANGES ASSOCIATED WITH
SELECTION IN AGRONOMIC CROPS. 14.076,
20.016*

BREEDING-METHODS

GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF DURUM AND COMMON WHEAT. 17.025

GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060

RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN. 23.017

BREEDING-STOCKS

GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008

BREMIA-LACTUCAE

ORGANIZATION AND STABILITY OF GENES FOR RESISTANCE AND AVIRULENCE. 67.013 VARIATION IN LETTUCE DOWNY MILDEW. 12.008 BRITTLE-2-MAIZE,

DISSOCIATION MUTAGENESIS OF THE SHRUNKEN-2 LOCUS OF MAIZE. 14.012

BROWN-STEM-ROT-(SOYBEANS)

SOYBEAN BROWN STEM ROT. 23.008

BYDV

RFLP METHODOLOGY FOR RELATEDNESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

C-M-PV-SEPEDONICUM

RECOMBINANT DNA APPROACH TO DETECTION AND STRAIN DIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009

CABBAGE

CRUCIFER DISEASES. 12.047

CALCIUM

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

STRUCTURE AND EXPRESSIDN DF PLANT GENES ENCODING CALMODULIN. 18.014

CALLUS-(PLANTS)

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS.

06.008

SOYBEAN BROWN STEM ROT. 23.008

CALLUS-CELLS

CELL SPECIFIC GENE EXPRESSION DURING
LATICIFER DIFFERENTIATION IN POPPY. 67.096
CALMODULIN

STRUCTURE AND EXPRESSION OF PLANT GENES ENCODING CALMODULIN. 18.014

CAPRIC-ACID

DEVELOPMENT OF NEW AND IMPROVED CROPS FOR WATER CONSERVATION IN ARID LANDS. 25.004

CARBON-DIOXIDE

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

CARBON-DIOXIDE-FIXATION

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

CARBON-ISOTOPES

GLYPHOSATE DEGRADATION AND METABOLISM IN MICRODRGANISMS. 23.026, 36.001*

CARBON-METABOLISM

AMINO ACID METABOLISM AND THE RHIZOBIUM-LEGUME SYMBIOSIS. **66.014**, **67.107***

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010, 67.104 CAROTENOIDS

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIDS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

CARROTS

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNDS IN HIGHER PLANTS.

67 045

CATABOLISM

AMINO ACIO METABOLISM AND THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014.

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*. 17.018*

CAULIFLOWER-MOSAIC-VIRUS

DEFINING AND MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055* REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061

MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO. 65.001, 67.012*

CDNA-CLONES

RFLP AND MOLECULAR ANALYSIS OF ROOT KNOT NEMATODES, NEMATODE INFECTED PLANTS AND PEACHES. 10.009, 23.054*

CELERY

GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011

CELL WALLS OF MAIZE PERICARP. 15.010 CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE. 27.003

CELL-BIOLOGY

CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006

MOLECULAR MAPPING OF GENES IN CORN. 14.040

CELL-CULTURE

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010

CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048

EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. 65.004, 67.040* SOYBEAN BROWN STEM ROT. 23.008

THE ROLE OF POLYAMINES DURING STRESS-INDUCED ALTERATIONS IN GENE EXPRESSION. 67.073

TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*

CELL-GROWTH

CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010

CELL-LINES

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

MITOCHONORIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. 67.030

CELL-MEMBRANE

GENES FOR PHOTOSYNTHESIS IN CORN. 67.058 REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037

CELL-NUCLEUS

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003

CELL-PHYSIOLOGY

STRUCTURE AND EXPRESSION OF PLANT GENES ENCODING CALMODULIN. 18.014

CELLULAR

CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE. 27.003

CELLULAR-GENETICS

CELLULAR AND MOLECULAR GENETICS FOR CROP

IMPROVEMENT. 67.027, 67.035

CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002 GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010

CELLULASE

CELLULASE GENE EXPRESSION OURING FRUIT OEVELOPMENT. 67.004

MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUCOMONAS SPECIES. 67.034

CELLULASES

MOLECULAR BIOLOGY OF CELLULOSE AND LIGNIN OEGRADATION BY PHANEROCHAETE CHRYSOSPORIUM. 06.017

CENOCOCCUM-GEOPHILUM

RESEARCH ON THE INTRASPECIFIC VARIATION IN CENOCOCCUM GEOPHILUM FR.. 67.076

CEREALS

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.028

CELLULAR REGULATION OF THE EXPRESSION OF DORMANT GENES IN CEREAL GRAINS. 62.002

CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007

CHARACTERIZATION

CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF DS ELEMENT. 14.053

CHARACTERIZATION OF PLANT GENES ENCOOING PHOTOSYNTHETIC MEMBRANE PROTEINS. 67.074

CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES COOING FOR SPECIFIC GENES. 67.038

CHEMICAL-CONTROL-(INSECTS)

METHOOS OF CONTROLLING CORN INSECTS. 14.024

CHEMICAL-CONTROL-(PARASITES)

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

CHEMOTAXONOMY

INHERITANCE OF MITOCHONORIAL ONA IN SOMACLONAL VARIANTS. 20.003

CHENOPODIUM-QUINOA

GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPOOIUM). 14.073

CHICKPEAS

BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006*

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF F000 LEGUMES. 12.043, 62.007*

4

CHILLING

CHILLING AND GENE EXPRESSION IN FRUIT. 12.006 CHIMERAS

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014, 21.015

GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013

CHITINASE

CONTROL OF GENE EXPRESSION IN POTATO. 11.002 CHLAMYDOMONAS

BIOCHEMICAL AND OEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

CHLAMYDOMONAS-REINHARDTII

THE REPLICATION OF CHLOROPLAST ONA IN CHLAMYDOMONAS REINHARDII. 67.057 THE THYLAKOIO ENERGY TRANSDUCING ATPASE COMPLEX. 67.111

CHLORATES

MOLECULAR AND GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034

CHLOROPHYLL-BIOSYNTHESIS

HYBRIO VARIEGATION IN PHASEOLUS VULGARIS. 23.038

CHLOROPHYLLS

CHARACTERIZATION OF PLANT GENES ENCODING PHOTOSYNTHETIC MEMBRANE PROTEINS. **67.074**DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. **23.004**

FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. 67.020

HYBRID VARIEGATION IN PHASEOLUS VULGARIS. 23.038

CHLOROPLAST

SYSTEMATICS OF POTATOES AND THEIR WILD RELATIVES (SOLANUM SECT. PETOTA). 11.017

CHLOROPLAST-DEVELOPMENT

REPEATED ONA SEQUENCES AND CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008

THE REPLICATION OF CHLOROPLAST DNA IN CHLAMYDOMONAS REINHAROII. 67.057

CHLOROPLASTS

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH DXYGEN EVOLUTION. 67.087

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010

CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIOS AND CYBRIOS OF TOMATO. 12.030

DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS.

06.008

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. 67.020

GENES FOR PHOTOSYNTHESIS IN CORN. 67.058
GENETIC MECHANISMS IN CORN. 14.044

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. **06.009**

HYBRIO VARIEGATION IN PHASEOLUS VULGARIS. 23.038

INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE AND JACK PINES. **06.004**

MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020

MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. 67.089

ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. 20.005

PHOTOREGULATED GENE EXPRESSION IN CHLOROPLASTS. 67.081, 67.082

PLANT GENE REGULATION DURING NITROGEN ASSIMILATION. 67.075

THE REPLICATION OF CHLOROPLAST ONA IN CHLAMYDOMONAS REINHAROII. 67.057

THE THYLAKOIO ENERGY TRANSDUCING ATPASE COMPLEX. 67.111

TISSUE CULTURE ANO THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

CHLOROTIC-MOTTLE-VIRUS

THE NATURE OF RESISTANCE TO PLANT VIRUSES.

12.045 CHOCOLATE-SPOT

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

CHRISTMAS-TREES

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009

CHROMATIN

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008, 14.010

REGULATION OF PLANT GENE EXPRESSION BY ONA METHYLATION. 67.110

REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061

CHROMOPLASTS

MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. 67.089

CHROMOSOMAL-CONSTITUTIONS

BARLEY GENETICS AND PLANT CYTOGENETICS.

18.008, 20.004*, 27.001*, 67.022*

CHROMOSOMAL-INTEGRATION

TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

CHROMOSOMAL-VARIANTS

GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*

CHROMOSOME

DEVELOPMENT OF A MOLECULAR CYTOGENETIC MAP OF COTTON BY IN SITU HYBRIOIZATION. 21.012

CHROMOSOME-ABERRATIONS

DEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. 67.024

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.056

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001, 21.016

CHROMOSOME-BANDING

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. **06.016**

PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016

CHROMOSOME-DOUBLING

GENOME EVOLUTION IN BRASSICA. 12.012 SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

CHROMOSOME-EXCHANGE

DEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. **67**.**024**

RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN. 23.017

TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*

CHROMOSOME-HOMOLOGIES

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*

CHROMOSOME-LOSS

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.056

CHROMOSOME-MAPPING

CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. **06.011**

CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIDIZE TO UNIQUE-SEQUENCE-ONA CLONES. 17.031

CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034

GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006
GENETIC CONTROL OF SEMIGAMY; AND DERIVATION
OF NULLISOMIC COTTON. 21.013

GENETICS, CYTOGENITCS OF COTTON GERMPLASM.

```
21.008
```

GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001

MOLECULAR MAPPING OF GENES IN CORN. 14.040 ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017

THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001

TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

CHROMOSOME-MARKERS.

EXPLORATORY RESEARCH ON THE CYTOGENETICS OF SOLANACEOUS CROPS. 11.001

CHROMOSOME-PAIRING

GENETIC MECHANISMS IN CORN. 14.044

CHROMOSOME-SUBSTITUTION

GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY RECIPROCAL CHROMOSOME SUBSTITUTIONS.

TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

CHROMOSOMES

ANEUPLOID ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007

BACTERIAL GENES CODING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098

BARLEY GENETICS AND PLANT CYTOGENETICS.

18.008, 20.004*, 27.001*, 67.022*

BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033 CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*
CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT

HYBRIOIZE TO UNIQUE-SEQUENCE-ONA CLONES. 17.031

CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046 CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002
DEVELOPMENT OF A CYTOGENETIC MANIPULATION

SYSTEM FOR COTTON. 21.002

DEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. 67.024

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034, 23.056

GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOID WHEAT AND ITS RELATIVES. 67.097

GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF OURUM AND

COMMON WHEAT. 17.025 GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006 GENETIC CONTROL OF SEMIGAMY AND OERIVATION OF

NULLISOMIC COTTON. 21.014, 21.015 GENETIC MECHANISMS IN CORN. 14.044

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015 GENETICS OF FUNGAL PLANT PATHOGENS. 67.051

GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008

GENOME EVOLUTION IN BRASSICA. 12.012 GLIADIN GENES OF COMMON WHEAT AND ITS

ANCESTORS. 17.011
ISOLATION OF GENES THAT ENCODE DNA BINDING PROTEINS. **67.066**

ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATOOE MELOIDOGYNE INCOGNITA. 67.053 MAPPING THE SYMBIOSIS GENES OF PEA. 67.077 MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011 MOLECULAR MANIPULATION OF GENES. 28.002

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1 1 6

MOLECULAR MAPPING OF GENES IN CORN. 14.040 ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013

ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077

PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001, 21.016

REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061

STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE & THEIR USE AS RESEARCH TOOLS. 14.018

SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

USE OF SINGLE COPY CLONED DNA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028 VEGETABLE GENETICS. 12.014

CICER

GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

CINNAMIC-ACID

PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. 67.041

CINNAMYL-ALCOHOL-DEHYDROGENASE

ISOLATION OF A LIGNIN-BIOSYNTHESIS GENE FROM LOBLOLLY PINE. 06.010

CITRUS

CELLULAR GENETICS OF CITRUS SPECIES. 09.002 CLASSIFICATION-SYSTEMS

MEASURING THE GENETIC DIVERSITY OF CULTIVATEO PLANTS USING ISOZYME FREQUENCIES. 14.060

CLONES

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.027

CHILLING AND GENE EXPRESSION IN FRUIT. 12.006 DEVELOPMENT OF A DNA HYBRIOIZATION ASSAY FOR DETECTION OF THE RING ROT PATHOGEN. 11.007

DEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.056

GENETIC MANIPULATION OF LACTIC OEHYOROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019

GENETIC REGULATION OF A SEEO-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*
GENETIC REGULATION OF THE SOYBEAN UREASE

ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*

GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS. 14.057 MOLECULAR AND GENETIC STUDIES OF NITRATE

REOUCTASE IN BARLEY. 18.034 MOLECULAR SWITCHES IN PLASTID

OIFFERENTIATION. 67.089

ORGANIZATION AND CONTROL OF THE HYDROLASE GENES IN BARLEY. 18.018

ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017

REGULATION OF GENE EXPRESSION IN PLANTS.

14.020

REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003

RFLP METHOOOLOGY FOR RELATEONESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

THE PHYCOCYANIN GENES OF AGMENELLUM QUAORUPLICATUM. 67.088

CLONING

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.013, 26.002*, 67.028

CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002

CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013

CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010

CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. 67.014

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING DIHYDROFOLATE REDUCTASE. 23.053

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

EXPLOITATION OF RIBOSOMAL ONA TO CONTROL PLANT PATHOGENIC FUNGI. 67.101

GENE EXPRESSION IN PLANTS: A STUDY OF POLYADENYLATION IN PLANTS. 20.007

GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027

GENETICS AND CYTOLOGY OF MAIZE. 14.022
GENETICS OF NITROGEN FIXATION IN AZOTOBACTER
VINELANDII. 23.048, 23.049, 66.006*,

GLYPHOSATE DEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113

ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003

MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO. 65.001, 67.012*

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069

MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEU00MONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006

MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067

MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA DUCH.. 10.005

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047

PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH DNA METHYLATION. 23.028 REGULATION OF TUBER PROTEIN SYNTHESIS IN

POTATO. 11.014, 67.094*

REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNDS IN HIGHER PLANTS. 67.045

TOWAROS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

TRANSPOSABLE ELEMENTS IN MUTATOR ACTIVITY IN MAIZE. 14.026

CLOSTRIDIUM-PASTEURIANUM

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIDIUM PASTEURIANUM AND OTHER

MICROORGANISMS. 67.103

CLOVER

REPEATEO ONA SEQUENCES AND CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008

CLUSTER-ANALYSIS

ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA ANO OTHER EUROPEAN COUNTRIES. 14.080

MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021

COCHLIOBOLUS-HETEROSTROPHUS

GENETICS OF FUNGAL PLANT PATHOGENS. **67.051** COLCHICINE

GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013

COLD-HARDINESS

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.027

COLD-TOLERANCE

GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY RECIPROCAL CHROMOSOME SUBSTITUTIONS.

17.024

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009

COLLARDS

GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011

COLLETOTRICHUM

BIOCHEMISTRY OF PLANT CUTICLE. 67.108

COLLETOTRICHUM-GLOEOSPORIOIDES

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. **09.004**

COLONIZATION

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

COMPARATIVE-ANALYSIS

CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. **67.014**THE REPLICATION OF CHLOROPLAST DNA IN CHLAMYDOMONAS REINHARDII. **67.057**

COMPLEMENTARY-DNA

BIOCHEMISTRY OF PLANT CUTICLE. 67.108
CELLULAR REGULATION OF THE EXPRESSION OF DORMANT GENES IN CEREAL GRAINS. 62.002

CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. 67.014

EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 17.023*, 18.029*, 67.104

ISOLATION ANO CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059

ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA. 67.069

MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067

MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067

ORGANIZATION AND CONTROL OF THE HYDROLASE GENES IN BARLEY. 18.018

ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. **62.001**

THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN AND COTTON. 14.068, 21.007*

THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION ANO EXPRESSION. 18.036, 63.007* VARIATION IN LETTUCE DOWNY MILDEW. 12.008

COMPLEMENTATION

HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113

COMPLEXES

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE, 14.077

COMPUTER-TECHNIQUES

ORGANIZATION AND CONTROL OF THE HYDROLASE GENES IN BARLEY. 18.018

CONFORMATION

THE THYLAKOIO ENERGY TRANSDUCING ATPASE COMPLEX. 67.111

CONIFERS

CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. 06.016
OEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR

GENETIC IMPROVEMENT OF WOODY PLANTS. 06.008

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. 06.013

CONJUGATION

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*

CONSERVATION-TILLAGE

METHOOS OF CONTROLLING CORN INSECTS. 14.024

ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEDLING. 14.070

CONTROL-TECHNIQUES

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*

CORN

A PLANT MITOCHONORIAL MATURASE GENE. 67.099 ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. 67.043

BIOCHEMICAL AND DEVELOPMENTAL GENETICS OF HIGHER PLANTS. 14.038, 18.020

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033 BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING OISEASES OF CORN. 14.035. 15.005*

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF OS ELEMENT. 14.053

CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008, 14.010 CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046

CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. 67.014

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS ANO CYTOGENETICS. 14.043

CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015

OEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. 14.061

OEVELOPMENTAL GENETICS USING THE ALCHOL OEHYOROGENASE GENE-SYSTEM IN MAIZE. 14.004

DISSECTION OF HETEROSIS OF QUANTITATIVE

TRAITS USING MOLECULAR MARKERS. 14.056 EXPRESSION OF MAIZE MITOCHONORIAL GENOME. 14,002

GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027

GENES FOR PHOTOSYNTHESIS IN CORN. 67.058 GENETIC CONTROL OF LEAF LESION FORMATION IN MAIZE. 14.047

GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE.

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH ANO PRODUCTION EFFICIENCY. 06.018. 12.048*, 14.079*

GENETIC MECHANISMS IN CORN. 14.044 GENETICS AND CYTOLOGY OF MAIZE. 14.022

GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS. 14.057 GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034.

15.004*, 17.018*
GENETICS OF ASPERGILLUS FLAVUS. 67.036 GENETICS OF FUNGAL PLANT PATHOGENS. 67.051 ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE FUNCTION AND REGULATION. 14.054

0 1

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059

ISOLATION OF TRANSPOSON INOUCEO MAIZE MUTANTS OFFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACID. 14.001

ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058. 14.063

ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080

MAIZE GRAIN PROTEIN COMPOSITION AND **OISTRIBUTION AS RELATEO TO HARDNESS,** HANOLING AND PROCESSING. 14.016

MEASURING THE GENETIC OIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060 METHOOS OF CONTROLLING CORN INSECTS. 14.024

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069

MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011 MOLECULAR CLONING OF THE GLUTATHIONE

S-TRANSFERASE GENES FROM CORN. 14.067

MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*

MOLECULAR MAPPING OF GENES IN CORN. 14.040 MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021 MUTANT GENES THAT AFFECT ENOOSPERM

OEVELOPMENT IN MAIZE. 14.078

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077

RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*

REGULATION OF GENE EXPRESSION IN PLANTS. 14.020

STRUCTURE OF GENES INVOLVED IN DIL SYNTHESIS IN MAIZE. 14.072

STRUCTURE. VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036 STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE

& THEIR USE AS RESEARCH TOOLS. 14.018

SYSTEMS FOR THE MOLECULAR ANALYSIS OF OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN

AND COTTON. 14.068, 21.007*
TISSUE-SPECIFIC GENE REGULATION IN MAIZE.
14.050

TUBULIN GENES OF PLANTS. 67.060

CORN-OIL

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIOANTS IN CORN. 14.014

GENETIC ENGINEERING OF DILSEED SPECIES TO IMPROVE DIL QUALITY. 23.020, 25.003*

STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS IN MAIZE. 14.072

CORN-STALKS

METHOOS OF CONTROLLING CORN INSECTS. 14.024 CORN.

OISSOCIATION MUTAGENESIS OF THE SHRUNKEN-2 LOCUS OF MAIZE. 14.012

CORRELATION

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008

CORYNEBACTERIUM-SEPEDONICUM

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010

DEVELOPMENT OF A DNA HYBRIDIZATION ASSAY FOR DETECTION OF THE RING ROT PATHOGEN. 11.007

RECOMBINANT ONA APPROACH TO DETECTION AND STRAIN DIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009

COSMID

THE MOLECULAR BASIS OF BLACK ROT OF CRUCIFERS. 12.001

COSMIDS

CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012

COSMIO MAPPING OF MITOCHONORIAL ONA OF MALE FERTILE AND MALE STERILE SORGHUM. 15.002

MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013

COTTON

OEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002

OEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. 67.024

ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

GENETIC CONTROL OF SÉMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014, 21.015

GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003

GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001, 21.016

THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN AND COTTON. 14.068, 21.007*

COTTON-PESTS

POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS. 21.004 COTYLEDONS

GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

COUPLING-FACTOR

THE THYLAKOID ENERGY TRANSDUCING ATPASE COMPLEX. 67.111

COVARIANCE

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051

COWPEAS

CELLULAR METABOLISM IN PLANTS. 67.049
THE NATURE OF RESISTANCE TO PLANT VIRUSES.
12.045

CROP-LOSSES

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

CROP-PRODUCTION

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.027

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 17.023*, 18.029*

MAIZE GRAIN PROTEIN COMPOSITION AND OISTRIBUTION AS RELATED TO HARDNESS, HANOLING AND PROCESSING. 14.016

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

CROP-QUALITY

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIDS, AND NATURAL ANTIOXIOANTS IN CORN. 14.014

DEVELOPMENT AND USE OF NEAR ISOGENIC LINES

DEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELD AND GENE EXPRESSION. 67.018

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*

MAIZE GRAIN PROTEIN COMPOSITION AND DISTRIBUTION AS RELATED TO HARONESS, HANDLING AND PROCESSING. 14.016

CROP-VARIETIES

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060

CROP-YIELDS RIGHTS EPIDEMINING

BIOLOGY, EPIDEMIOLOGY, GENETICS, ANO CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

OEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELD AND GENE EXPRESSION. **67.018**

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE AND FOOD VALUE. 12.021

ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058

MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND DEVELOPMENT. 67.015

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS IN MAIZE. 14.072

SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

CROPS

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*

CROSSBREEDING

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

GENETIC STRUCTURE OF CULTIVATED SOYBEANS AND THE WILD SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045

STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. **14.036**

CROSSES

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC

MANIPULATIONS. 17.009
GENETIC CONTROL OF PLANT MORPHOGENESIS: BARLEY CHROMOSOMES. 18.007 CELLULAR GENETICS OF CITRUS SPECIES. 09.002 STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046 14.065 COSMIO MAPPING OF MITOCHONORIAL ONA OF MALE FERTILE AND MALE STERILE SORGHUM. 15.002 CROWN-GALLS CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*
REGULATION OF PLANT GENE EXPRESSION BY ONA LENGTH POLYMORPHISMS (RFLPS). 14.015 CYTOGENETIC MANIPULATIONS FOR ALFALFA METHYLATION. 67.110 IMPROVEMENT. 20.002 CRUCIFERAE CYTOGENETIC STUDIES OF HAROWOOD AND CRUCIFER DISEASES. 12.047 CONIFEROUS FOREST TREES. 06.016 CRYPTOCOCCUS-FAGISUGA GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006 GENETIC MOSAICS AND THEIR RELATIONSHIP TO GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF PATTERNS OF SUSCEPTIBILITY TO INSECTS AND NULLISOMIC COTTON. 21.014 OISEASES. **06.003** GENETIC MECHANISMS IN CORN. 14.044 CUCURBITA-PEPO GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015 GENETIC STRUCTURE OF WEED-CROP POPULATION GENETICS AND IMPROVEMENT OF CORN USING SYSTEMS (CUCURBITA AND CHENOPOOIUM). MOLECULAR AND TRADITIONAL METHODS. 14.057 14.073 GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018 **CULTIVARS** ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010 CLONING OF GENES FROM MUTANTS REGULATING GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF LYSINE IN CEREALS. 16.003, 17.019*, FOOO LEGUMES. 12.043, 62.007* 23.030*, 26.003* PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR ISOZYME LOCI AS MARKERS FOR LOCATING AND REGULA- TION OF OAT STORAGE PROTEINS. MANIPULATING QUANTITATIVE TRAIT LOCI. 18.035 14.063 RELATION OF ISOZYME LOCI TO QUALITATIVE ORGANIZATION OF ALPHA AMYLASE GENES IN CHARACTERS OF SOYBEAN. 23.017 BARLEY. 18.017 PHYSICAL MAPPING OF THE GENOME OF WHEAT. TOWARDS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017 17.016 CULTIVATED-PLANTS PRESERVATION AND UTILIZATION OF GERMPLASM IN 18.003, 67.006* COTTON. 21.001, 21.016 RELATION OF ISOZYME LOCI TO QUALITATIVE MEASURING THE GENETIC DIVERSITY OF CULTIVATEO PLANTS USING ISOZYME FREQUENCIES. 14.060 CHARACTERS OF SOYBEAN. 23.017 MOLECULAR GENETIC EVALUATION OF PLANT TUBULIN GENES OF PLANTS. 67.060 GERMPLASM RESOURCES. 67.017 VEGETABLE GENETICS. 12.014 CULTURAL-PRACTICES CYTOKININS BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & CONTROL OF GENE EXPRESSION IN POTATO. 11.002 MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR OF CORN. 14.035, 15.005* REGULA- TION OF OAT STORAGE PROTEINS. CUPHEA 18.035 DEVELOPMENT OF NEW AND IMPROVED CROPS FOR CYTOLOGICAL-ABERRATIONS BARLEY GENETICS AND PLANT CYTOGENETICS. WATER CONSERVATION IN ARID LANDS. 25.004 CUTICLE 18.008, 20.004*, 27.001*, 67.022* CYTOPLASM BIOCHEMISTRY OF PLANT CUTICLE. 67.108 CYTOPLASMIC FACTORS OF THE POTATO. 11.012 CUTIN BIOCHEMISTRY OF PLANT CUTICLE. 67.108 GENETICS AND MOLECULAR BIOLOGY OF COTTON **CUTINASES** CYTOPLASMIC MALE STERILITY. 21.003 BIOCHEMISTRY OF PLANT CUTICLE. 67.108 ORGANELLE DNA ORGANIZATION AND CYTOPLASMIC PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF MALE STERILITY IN PENNISETUM. 20.005 TROPICAL FRUIT AND THEIR CONTROL. 09.004 CYTOPLASMIC-GENETICS CY, CODING AND REGULATION OF MITOCHONDRIAL GENES. GENETIC/MOLECULAR CHARACTERIZATION OF THE 14.052 MUTATOR-RELATED CY TRANSPOSABLE ELEMENT CYTOPLASMIC-INHERITANCE CONSTRUCTION AND CHARACTERIZATION OF SOMATIC SYSTEM OF MAIZE. 14.031 HYBRIDS AND CYBRIDS OF TOMATO. 12.030 CYANO-BACTERIA STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046 COSMIO MAPPING OF MITOCHONDRIAL DNA OF MALE FERTILE AND MALE STERILE SORGHUM. 15.002 STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED DURING INHERITANCE OF MITOCHONDRIAL DNA IN NITROGEN FIXATION. 67.084 SOMACLONAL VARIANTS. 20.003 THE PHYCOCYANIN GENES OF AGMENELLUM CYTOPLASMIC-MALE-STERILITY ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA QUADRUPLICATUM. 67.088 CYSTEINE MAYS MTONA. 67.043 ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, CONSTRUCTION AND CHARACTERIZATION OF SOMATIC 23.030*, 26.003* CYTOCHROMES HYBRIDS AND CYBRIDS OF TOMATO. 12.030 PLANT CYTOCHROMES P-450 REGULATION OF GENE ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE EXPRESSION. 67.041 SEEOLING. 14.070 STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046 GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOGENETICS

1

6 1

CYTOPLASMIC MALE STERILITY. 21.003

ANEUPLOIO ANALYSIS OF GENETIC ARCHITECTURE OF

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005 ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. 20.005

CYTOPLASMIC-VARIABILITY

GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

CYTOTAXONOMY

CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. 06.016

DAIRY-PRODUCTS

GENETIC IMPROVEMENT OF PROPIONIBACTERIA ANO OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*

DATA

ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA ANO OTHER EUROPEAN COUNTRIES. 14.080

DATA-ANALYSIS

GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND DISEASES. 06.003

ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080

DEFENSE-MECHANISMS

ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

DEGRADATION

CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*

DEHYDROGENASES

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

DELETIONS

GENETIC ANALYSES OF THE MUTATOR SYSTEM OF MAIZE. 14.028

DEPENDENCY

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

DETECTION

RECOMBINANT DNA APPROACH TO OETECTION AND STRAIN DIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009

DETECTION.

BASIC AND APPLIED MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN. 11.008

DEVELOPMENT

CELL WALLS OF MAIZE PERICARP. 15.010 ROLE OF LOW MOLECULAR WEIGHT HEAT SHOCK PROTEINS IN PLANT OEVELOPMENT. 67.002 DEVELOPMENT,

REGULATION OF PLANT GENE EXPRESSION. 67.106 DIFFERENTIATION

HYBRID VARIEGATION IN PHASEOLUS VULGARIS.

23.038

MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. 67.089

SYSTEMS FOR THE MOLECULAR ANALYSIS OF OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

DIHYDROFOLATE-REDUCTASE

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING OIHYDROFOLATE REDUCTASE. 23.053

DIPLOIDS

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*

GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOID WHEAT AND ITS RELATIVES. 67.097

GENETICS OF ASPERGILLUS FLAVUS. 67.036 GENOME EVOLUTION IN BRASSICA. 12.012 ORGANIZATION OF ALPHA AMYLASE GENES IN

DISEASE-CONTROL

BARLEY. 18.017

ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS.

12.024, 16.005*, 23.032*, 63.003* GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004

DISEASE-DETECTION

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010 OEVELOPMENT OF A ONA HYBRIOIZATION ASSAY FOR DETECTION OF THE RING ROT PATHOGEN. 11.007

DISEASE-DIAGNOSIS

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010 PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047

PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

DISEASE-MUTAGENESIS

THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIDASE. 26.005

DISEASE-RESISTANCE

18.003, 67.006*

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.041

VARIATION IN LETTUCE DOWNY MILDEW. 12.008

DISEASE-RESISTANCE-GENES

TAGGING PLANT GENES WITH TIGHTLY-LINKED RFLP MARKERS. 12.035

DISEASE-RESISTANCE,

ORGANIZATION AND STABILITY OF GENES FOR RESISTANCE AND AVIRULENCE. 67.013

DISEASE-SURVEYS

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

DISEASE-SUSCEPTIBILITY

CYTOPLASMIC FACTORS OF THE POTATO. 11.012 GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND DISEASES. 06.003

PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

DISEASE-VECTORS

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

```
DISPERSAL
```

POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS. 21.004

DISTRIBUTION-PATTERNS

GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007,

DNA

18.003. 67.006*

- A STUDY OF GENETIC DIVERSITY IN PEANUTS USING CHLOROPLAST AND RRNA MOLECULAR MARKERS.
- ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003
- ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010
- ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. 67.043
- BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033
- BIOCHEMISTRY OF PLANT CUTICLE. 67.108
- CELL SPECIFIC GENE EXPRESSION OURING LATICIFER OIFFERENTIATION IN POPPY. 67.096
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010, 67.027
- CELLULASE GENE EXPRESSION OURING FRUIT OEVELOPMENT. 67.004
- CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007
- CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012
- CHARACTERIZATION OF NITROGEN FIXATION GENES AND THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057
- CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES COOING FOR SPECIFIC GENES. 67.038
- CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. 06.011
- CHROMATIN STRUCTURE AND GENE EXPRESSION IN
- MAIZE. 14.010 CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014
- CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*
- CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. 67.014
- CONTROL OF GENE EXPRESSION IN POTATO. 11.002 OEVELOPMENT AND USE OF NEAR ISOGENIC LINES
- FOR GENETIC ANALYSIS OF YIELO AND GENE EXPRESSION. **67.018**OEVELOPMENT OF A ONA HYBRIOIZATION ASSAY FOR
- OETECTION OF THE RING ROT PATHOGEN. 11.007
- OEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. 14.061
- DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS. 06.008
- OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*
- OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015
- ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEOLING. 14.070
- ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039
- EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051
- EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*
- EXPLOITATION OF RIBOSOMAL ONA TO CONTROL

- PLANT PATHOGENIC FUNGI. 67.101 FUNCTION OF PLANT RNA-OEPENOENT RNA POLYMERASES. 12.036
- GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033 GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034, 23.056
- GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007.
- GENETIC MANIPULATION OF LACTIC DEHYDROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE.

- 1

- GENETICS AND CYTOLOGY OF MAIZE. 14.022 GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009
- GENETICS OF FUNGAL PLANT PATHOGENS. 67.051 GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018
 GLYPHOSATE OEGRADATION AND METABOLISM IN
- MICROORGANISMS. 23.026, 36.001*
 HOST-PATHOGEN RECOGNITION AND DISEASE
- RESISTANCE IN PLANTS. 67.113
- HYBRIO VARIEGATION IN PHASEOLUS VULGARIS. 23.038
- INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE ANO JACK PINES. 06.004
- INHERITANCE OF MITOCHONORIAL ONA IN
- SOMACLONAL VARIANTS. 20.003
 ISOLATION OF GENES THAT ENCODE ONA BINOING PROTEINS. **67.066**
- MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004. 23.005*
- MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO. 65.001, 67.012*
- MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*
- MOLECULAR MAPPING OF GENES IN CORN. 14.040 MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM OIVERSITY AND SYSTEMATICS. 12.020
- MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. 67.089
- ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. 20.005
- ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. 62.001
- ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055
- ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013
- ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017
- PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. 67.041
- PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075
- PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028
- PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.016
- REGULATION OF PLANT GENE EXPRESSION BY ONA METHYLATION. 67.110
- REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSION. 23.001
- REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNOS IN HIGHER PLANTS. 67.045
- REPEATED ONA SEQUENCES AND CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008
- REVERSE TRANSCRIPTASE AND REPLICATION OF

```
CAULIFLOWER MOSAIC VIRUS. 67.061
  STRUCTURE AND EXPRESSION OF PLANT GENES
     ENCOOING CALMOOULIN. 18.014
  STRUCTURE AND REGULATION OF NIF GENES IN
     RHIZOBIUM FREOII, A NEWLY OESCRIBEO
     SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*
  SYSTEMATICS OF POTATOES AND THEIR WILD
  RELATIVES (SOLANUM SECT. PETOTA). 11.017
THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007
  THE NATURE OF RESISTANCE TO PLANT VIRUSES.
  THE PHYCOCYANIN GENES OF AGMENELLUM
  QUAORUPLICATUM. 67.088
TISSUE CULTURE AND THE TRANSFER OF
     CYTOPLASMIC GENES. 17.027, 18.031*
  TISSUE CULTURE GENETIC SYSTEMS. 14.041,
     18.022*
  WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS.
     17.006, 67.021*
DNA-CLONING
  ANALYSIS OF SEED GLOBULIN GENES AND THEIR
     EXPRESSION IN CEREALS. 18.016
  BIOCHEMISTRY OF PLANT CUTICLE. 67.108
  CELLULAR AND MOLECULAR GENETICS FOR CROP
 IMPROVEMENT. 18.010, 67.035
CHARACTERIZATION OF THE ROLE OF A STRESS
     PROTEIN (GENE(S) IN SALT WATER STRESS
     TOLERANCE IN PLANTS. 67.048
  CHILLING AND GENE EXPRESSION IN FRUIT. 12.006
  CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT
     HYBRIOIZE TO UNIQUE-SEQUENCE-DNA CLONES.
     17.031
  CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT
     LENGTH POLYMORPHISMS (RFLPS). 14.015
  OEFINING AND MAPPING THE GENES OF
     CAULIMOVIRUSES. 66.004, 67.055*
  GENETIC ENGINEERING TO IMPROVE PLANT HEALTH
     AND PRODUCTION EFFICIENCY. 12.010, 67.104
  ISOLATION AND CHARACTERIZATION OF GENES
     REGULATEO BY LIGHT. 67.080
  MECHANISMS DIRECTING HORMONAL AND
     OEVELOPMENTAL REGULATION OF GENE
     EXPRESSION IN BARLEY. 18.026
  MOLECULAR AND GENETIC BASIS OF PATHOGENESIS
     OF PSEUDOMONAS SPECIES. 67.034
 MOLECULAR AND GENETIC STUDIES OF NITRATE
     REOUCTASE IN BARLEY. 18.034
  MOLECULAR CHARACTERIZATION OF THE SUCROSE
     SYNTHETASE-2 GENE OF MAIZE. 14.011
  ORGANIZATION AND EXPRESSION OF A-AMYLASE
     GENES IN THE BARLEY GENOME. 62.001
  ORGANIZATION AND EXPRESSION OF
     LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS
     GLUTINOSA. 67.092
  PATHOGENESIS AND RELATIONSHIPS OF PLANT
     VIRUSES. 67.047
  PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL
     CONTROL OF XANTHOMONAD DISEASES OF FRUIT
     TREES. 09.006, 10.004*, 66.005*
  PHOTOREGULATEO GENE EXPRESSION IN
     CHLOROPLASTS. 67.082
  PLANT GENE REGULATION DURING NITROGEN
     ASSIMILATION. 67.075
  PLASMIDS IN PLANT PATHOGENIC BACTERIA.
     18.012, 26.001*
  STRUCTURE AND EXPRESSION OF SOYBEAN LEAF
     STORAGE PROTEIN GENES. 23.043
  THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION
    ANO EXPRESSION. 18.036, 63.007*
  USE OF SINGLE COPY CLONED DNA SEQUENCES AS
     GENETIC MARKERS IN BARLEY. 18.028
  VARIATION IN LETTUCE DOWNY MILDEW. 12.008
  VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED
```

CELLS. 67.054

DNA-FRAGMENTS CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIOIZE TO UNIQUE-SEQUENCE-ONA CLONES. CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015 CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002 DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056 ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039 GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034 GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018* HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002 MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUOOMONAS SPECIES. 67.034 MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022 MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020 DNA-HYBRIDIZATION ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. **67.043** CHARACTERIZATION OF PLANT GENES ENCOOING PHOTOSYNTHETIC MEMBRANE PROTEINS. 67.074 CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES COOING FOR SPECIFIC GENES. 67.038 GENE REGULATION. EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007. 15.001* GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027 ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. **67.092** ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017 RECOMBINANT DNA APPROACH TO DETECTION AND STRAIN DIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009 REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003 REPEATED ONA SEQUENCES AND CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008 WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021* DNA-INSERTION CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006 MOLECULAR MANIPULATION OF GENES. 28.002 VIRAL GENE EXPRESSION IN POTYVIRUS INFECTEO CELLS. 67.054 DNA-POLYMERASE REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061 **DNA-PROBES** RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN SORGHUM. 15.009 DNA-REPLICATION REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061 THE REPLICATION OF CHLOROPLAST ONA IN CHLAMYDOMONAS REINHARDII. 67.057

DNA-SEQUENCES
A COMPARATIVE STUDY OF N(2)-FIXING GENES IN

TISSUE CULTURE GENETIC SYSTEMS. 14.041,

18.022*

- CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. **67.103**
- ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003
- ANALYSIS OF SEEO GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016
- ANALYSIS OF THE OIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015
- ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. **67.043**
- CELLULAR ANO MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010, 67.028
- CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002
- CELLULASE GENE EXPRESSION OURING FRUIT OEVELOPMENT. 67.004
- CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012
- CHARACTERIZATION OF NITROGEN FIXATION GENES ANO THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057
- CHARACTERIZATION OF PLANT GENES ENCOOING PHOTOSYNTHETIC MEMBRANE PROTEINS. **67.074**
- CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013 CHARACTERIZATION OF THE MAIZE GENOME:
- CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES COOING FOR SPECIFIC GENES. 67.038
- CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. **06.011**
- CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048
- CHROMATIN STRUCTURE ANO GENE EXPRESSION IN MAIZE. 14.010
- CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*
- CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIOIZE TO UNIQUE-SEQUENCE-ONA CLONES. 17.031
- CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. **16.006**
- CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEOLING OIHYOROFOLATE REOUCTASE. 23.053
- CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015
- OEFINING ANO MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*
- OEVELOPMENTAL GENETICS USING THE ALCHOL OEHYOROGENASE GENE-SYSTEM IN MAIZE. 14.004
- OEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004
- ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEOLING. 14.070
- EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*
- EXPLOITATION OF RIBOSOMAL ONA TO CONTROL PLANT PATHOGENIC FUNGI. 67.101
- EXPRESSION OF MAIZE MITOCHONORIAL GENOME. 14.002
- FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. 67.020
- GENE EXPRESSION IN PLANT OEVELOPMENT. 23.033 GENE MAPPING IN SOYBEAN WITH MOLECULAR
- MARKERS. 23.034
 GENE STRUCTURE AND EXPRESSION IN PLANTS.
- GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11 004
- GENOME ORGANIZATION IN THE CULTIVATEO ANO

- WILO SPECIES OF TOMATO. 12.015 Gliadin genes of common wheat and its ancestors. 17.011
- ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE FUNCTION AND REGULATION. 14.054
- ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059
- ISOLATION AND CHARACTERIZATION OF GENES REGULATED BY LIGHT. **67.080**MECHANISM OF ACTION OF PLANT GROWTH
- MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND OEVELOPMENT. 67.015
- MECHANISMS OIRECTING HORMONAL AND OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026
- MITOCHONDRIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. **67.030**
- MITOCHONDRIAL GENOME FUNCTION IN CYTOPLASMIC
 MALE STERILE AND FERTILE PLANTS. 62.005
 MORILE ELEMENTS IN THE SOUREAN GENOME 23.03
- MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036
 MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN
 ANTHERS OF BRASSICA. 67.069
- MOLECULAR ANO GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034
- MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA.
- 23.002, 66.001*

 MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011
- MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067
- MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003
- MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. **67.067**
- MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*
- MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017
- MOLECULAR MANIPULATION OF GENES. 28.002 MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047
- ORGANIZATION AND CONTROL OF THE HYOROLASE GENES IN BARLEY. 18.018
- ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. **62.001**
- ORGANIZATION ANO EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. 67.092
- PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075
- PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028
- RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*. 18.030*. 67.085*
- 17.026*, 18.030*, 67.085*
 REGULATION OF PLANT GENE EXPRESSION BY ONA METHYLATION. 67.110
- REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNOS IN HIGHER PLANTS. 67.045
- REPEATEO ONA SEQUENCES AND CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008
- STRUCTURE AND EXPRESSION OF PLANT GENES ENCODING CALMODULIN. 18.014
- STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043
- STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. **67.044**

STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036 THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN ANO COTTON. 14.068, 21.007* THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001 THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045 THE PHYCOCYANIN GENES OF AGMENELLUM QUAORUPLICATUM. 67.088 THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION ANO EXPRESSION. 18.036, 63.007* THE REPLICATION OF CHLOROPLAST DNA IN CHLAMYOOMONAS REINHAROII. 67.057 USE OF SINGLE COPY CLONEO ONA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028 VIRAL GENE EXPRESSION IN POTYVIRUS INFECTEO CELLS. 67.054 WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021* DNA-SEQUENCING A PLANT MITOCHONORIAL MATURASE GENE. 67.099 DNA-STRUCTURE 18.003, 67.006* CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.010 EXPLOITATION OF RIBOSOMAL ONA TO CONTROL PLANT PATHOGENIC FUNGI. 67.101 ISOLATION OF GENES THAT ENCODE ONA BINDING PROTEINS. **67.066** STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036 THE PHYCOCYANIN GENES OF AGMENELLUM QUADRUPLICATUM. 67.088 DNA-SYNTHESIS ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003 DNA-VECTORS CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*, CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013 GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVEO AGRONOMIC PROPERTIES. 23.039 MOLECULAR GENETIC APPROACHES FOR GERMPLASM DEVELOPMENT OF FRUIT AND NUT CROPS. 10.001 THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. 67.054 DNA, BASIC AND APPLIED MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN. 11.008 DOT-BLOT RECOMBINANT ONA APPROACH TO DETECTION AND STRAIN OIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009 DOWNY-MILDEW-(LETTUCE) VARIATION IN LETTUCE OOWNY MILOEW. 12.008 DOWNY-MILDEW. ORGANIZATION AND STABILITY OF GENES FOR

RESISTANCE AND AVIRULENCE. 67.013 DROSOPHILA TOWAROS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017 DROUGHT REGULATION OF PLANT GENE EXPRESSION BY CELL TURGOR OR ABSCISIC ACID. 67.093 DROUGHT-STRESS REGULATION OF GENE EXPRESSION BY ABSCISIC ACIO DURING DROUGHT STRESS. 67.016

ELECTROPHORES IS DROUGHT-TOLERANCE CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009 GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009 INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LODGEPOLE ANO JACK PINES. 06.004 MOLECULAR GENETIC APPROACHES FOR GERMPLASM OEVELOPMENT OF FRUIT AND NUT CROPS. 10.001 VEGETABLE GENETICS. 12.014 DROUGHT-TOLERANCECARBON-ISOTOP BARLEY BREEDING AND GENETICS. 18.027 DRY-PEAS BREEDING, DISEASES AND CULTURE OF DRY PEAS ANO LENTILS. 12.040, 62.006* DUPLICATION ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077 **DUPLICATION-DEFICIENCIES** OEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. 67.024 DURUM-WHEAT GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC ANO QUALITY CHARACTERISTICS OF DURUM AND COMMON WHEAT. 17.025 **ECHINACEA** EVALUATION OF GENETIC VARIABILITY IN ECHINACEA. 25.005, 28.003* **ECOLOGY** THE PHYTOPATHOGENIC BACTERIA. 67.052 ECONOMIC-THRESHOLDS METHOOS OF CONTROLLING CORN INSECTS. 14.024 ECOSYSTEMS ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010* EDIBLE-MUSHROOMS GERMPLASM ENHANCEMENT AND CULTURE OF EOIBLE MUSHROOMS. 12.038 **EFFICACY** IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIOES BY RECOMBINANT ONA TECHNOLOGY. 65.002, 67.031* PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011 **ELECTRON-MICROSCOPY** REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003 **ELECTRON-SPIN-RESONANCE** STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046 **ELECTROPHORESIS** 18.003.67.006* BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIOANTS IN CORN. **14.014** EFFICIENCY OF NITROGEN FIXATION. 23.025. 66.003* ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL ANO CHICKPEA USING ISOZYME ANO ONA MARKERS. 12.039, 12.041 EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005* EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT

GENETICS RESEARCH. 12.032 FURTHER OEVELOPMENT OF PROTEIN-SPECIFIC

GENETIC MARKERS IN TOMATO. 12.034

GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY

RECIPROCAL CHROMOSOME SUBSTITUTIONS.

17.024

GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOID WHEAT AND ITS RELATIVES. 67 097

GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005

GENETIC STRUCTURE OF CULTIVATEO SOYBEANS AND THE WILD SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045

GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011

GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISDZYME FREQUENCIES. 14.060

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. **06.013**

STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036 VEGETABLE GENETICS. 12.014

ELEMEN'

MOLECULAR ANALYSIS OF A SOYBEAN TRANSPOSABLE ELEMENT. 23.023

ELICITORS

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*

ELYTRIGIA

TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

EMBRYO

GENETIC ENGINEERING OF DILSEED SPECIES TO IMPROVE DIL QUALITY. 23.020, 25.003*

EMBRYO-CULTURE

VEGETABLE GENETICS. 12.014

EMBRYOGENESIS

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*

CHROMOSOMAL MAPPING OF GENES CONTROLLING
TISSUE CULTURE RESPONSE IN WHEAT. 17.010

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEDLING. 14.070

EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67 042*

EMBRYOS

EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*

ENDONUCLEASES

MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020

ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. 20.005

ENDOSPERM

CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012

GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005

MAIZE GRAIN PROTEIN COMPOSITION AND OISTRIBUTION AS RELATED TO HARONESS, HANDLING AND PROCESSING. 14.016

MUTANT GENES THAT AFFECT ENOOSPERM
DEVELOPMENT IN MAIZE. 14.078
ORGANIZATION AND MANIPULATION OF WHEAT

STORAGE PROTEIN GENES. 17.013
PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR
REGULA- TION OF DAT STORAGE PROTEINS.

18.035

ENERGY-TRANSFER

THE THYLAKOIO ENERGY TRANSOUCING ATPASE COMPLEX. 67.111

ENHANCEMENT

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

ENTOMOPHAGOUS-INSECTS

GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*, 21.005*, 23.040*

ENVIRONMENT

REGULATION OF ETHYLENE INDUCED GENE EXPRESSION OURING FRUIT RIPENING. **67.009**

ENVIRONMENTAL-FACTORS

GENETIC REGULATION OF A SEEO-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*
SOYBEAN BROWN STEM ROT. 23.008

ENVIRONMENTAL-INTERACTIONS

REGULATION OF ETHYLENE INDUCEO GENE EXPRESSION OURING FRUIT RIPENING. **67.009**

ENVIRONMENTAL-STRESS

ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS. 14.057

GENOME ORGANIZATION IN THE CULTIVATED AND WILD SPECIES OF TOMATO. 12.015

THE ROLE OF POLYAMINES OURING STRESS-INDUCED ALTERATIONS IN GENE EXPRESSION. 67.073

ENZYME-ACTIVITY

CELLULASE GENE EXPRESSION OURING FRUIT DEVELOPMENT. **67.004**

FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034 GENETICS OF NITRATE REDUCTION IN BARLEY.

67.105

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC
CO(2) ASSIMILATION, AND RUBISCO ACTIVITY
IN PLANTS. 12.023

ENZYME-CHARACTERIZATION

MOLECULAR ANO GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034

ENZYME-DEFICIENCY

MUTANT GENES THAT AFFECT ENDOSPERM DEVELOPMENT IN MAIZE. 14.078

ENZYME-FUNCTION

FUNCTION OF PLANT RNA-OEPENDENT RNA POLYMERASES. 12.036

ENZYME-INHIBITORS

NZYME-INHIBITURS

CLONING, EXPRESSION AND MUTAGENESIS OF

SOYBEAN SEEDLING OIHYOROFOLATE REOUCTASE.

23.053
DIFFERENTIAL EXPRESSION OF PROTEINASE
INHIBITOR GENES IN PLANT TISSUES. 11.015
REGULATION OF SOYBEAN PROTEIN GENE

EXPRESSION. 23.003 ENZYME-STRUCTURE

CELLULASE GENE EXPRESSION DURING FRUIT DEVELOPMENT. 67.004

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007

ENZYME-SYNTHESIS

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008

FUNCTION OF PLANT RNA-DEPENDENT RNA POLYMERASES. 12.036

GENETIC REGULATION OF THE SOYBEAN UREASE

ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*

ENZYME-SYSTEMS

ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015

ENZYMES

13,002

BACTERIAL GENES COOING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098 CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051

EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032

GENETIC STRUCTURE OF CULTIVATED SOYBEANS AND THE WILO SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003

GENETICS OF NITRATE REDUCTION IN BARLEY. 67.105

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006 MOLECULAR CLONING OF THE GLUTATHIONE

S-TRANSFERASE GENES FROM CORN. 14.067 MUTANT GENES THAT AFFECT ENOOSPERM

OEVELOPMENT IN MAIZE. 14.078 ORGANIZATION OF ALPHA AMYLASE GENES IN

BARLEY. 18.017 PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF

TROPICAL FRUIT AND THEIR CONTROL. 09.004 PLANT CYTOCHROMES P-450 REGULATION OF GENE

EXPRESSION. 67.041 RECOMBINANT ONA APPROACHES TO PLANT GENE

STRUCTURE AND GENOME OIVERSITY. 14.064, 17.026*, 18.030*, 67.085*

REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNOS IN HIGHER PLANTS. 67.045

REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN ANO COTTON: 14.068, 21.007*

EPIDEMIOLOGY

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010* CRUCIFER DISEASES. 12.047

EPIZOOTICS

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. 06.015

ERWINIA-CHRYSANTHEMI

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006 ESCHERICHIA-COLI

CHARACTERIZATION OF NITROGEN FIXATION GENES ANO THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057

EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*

GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANOII. 23.049, 66.007*

GLYPHOSATE OEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113

ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIDE BIOSYNTHESIS OF

RHIZOBIUM JAPONICUM. 23.027 THE PHYCOCYANIN GENES OF AGMENELLUM QUAORUPLICATUM. 67.088

ETHEPHON

REGULATION OF ETHYLENE INOUCEO GENE EXPRESSION OURING FRUIT RIPENING. 67.009

ETHREL

REGULATION OF ETHYLENE INOUCEO GENE EXPRESSION OURING FRUIT RIPENING. 67.009 **ETHYLENE**

MODULATION AND NITROGEN FIXATION OF PRC R.

JAPONICUM. 23.021

REGULATION OF ETHYLENE INDUCEO GENE EXPRESSION OURING FRUIT RIPENING. 67.009

ETIOLOGY

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

EVALUATION

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

EVOLUTION

18.003, 67.006*

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*

CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIDIZE TO UNIQUE-SEQUENCE-DNA CLONES. 17.031

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.041

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*

GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOIO WHEAT AND ITS RELATIVES. 67.097

GENOME EVOLUTION IN BRASSICA. 12.012, 12.013 ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. 06.013

REPEATED ONA SEQUENCES AND CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 USE OF MOLECULAR MARKERS IN PLANT BREEDING ANO GENETICS. 67.071

A PLANT MITOCHONORIAL MATURASE GENE. 67.099 **EXONS**

MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011

EXOTIC-BREEDS

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060 **EXOTIC-PLANTS**

GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS. 14.057 ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.063

EXOTIC-SPECIES

ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058

EXPRESSION

CELL WALLS OF MAIZE PERICARP. 15.010

EXTRA-CHROMOSOMAL-DNA

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*,

OEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. 14.061

EXTRA-NUCLEAR-INHERITANCE

BIOCHEMICAL AND DEVELOPMENTAL GENETICS OF HIGHER PLANTS. 14.038, 18.020

EXTREME-RESISTANCE

MOLECULAR GENETICS OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.005

FAGUS-GRANDIFOLIA

GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND OISEASES. 06.003

FATTY-ACID-MUTANTS

DEVELOPMENT OF NEW AND IMPROVED CROPS FOR WATER CONSERVATION IN ARIO LANOS. 25.004

FATTY-ACID-SYNTHESIS

CELLULAR METABOLISM IN PLANTS. 67.049 REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037

FATTY-ACIDS

REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037

FERMENTATION

GENETIC IMPROVEMENT OF PROPIONIBACTERIA ANO OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*

FERTILITY

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003 SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

FERTILIZATION

GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014, 21.015 GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013

CELLULAR AND MOLECULAR GENETICS FOR IMPROVEMENT OF MAIZE AND FESCUE. 14.071, 20.012*

FIELD-CROPS

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047

STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036 FIELDWORK

SYSTEMATICS OF POTATOES AND THEIR WILD RELATIVES (SOLANUM SECT. PETOTA). 11.017

FLAVANOIDS TISSUE-SPECIFIC GENE REGULATION IN MAIZE. 14.050

FLAVONOIDS

GENETIC MECHANISMS IN CORN. 14.044

FLOOD-RESISTANCE

OEVELOPMENTAL GENETICS USING THE ALCHOL OEHYOROGENASE GENE-SYSTEM IN MAIZE. 14.004

FLOOD-TOLERANCE

GENETIC MANIPULATION OF LACTIC DEHYDROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019

FLORAL-DEVELOPMENT

THE ROLE OF POLYAMINES OURING STRESS-INDUCED ALTERATIONS IN GENE EXPRESSION. 67.073

FLOUR-QUALITY

GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY RECIPROCAL CHROMOSOME SUBSTITUTIONS.

FOOD

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*

FOOD-LEGUMES

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF F000 LEGUMES. 12.043, 62.007*

FOOD-MICROBIOLOGY

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*

FOOD-MICROORGANISMS

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*

FOOD-NUTRITIVE-VALUE

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE ANO FOOO VALUE. 12.021

FOOD-QUALITY

GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF DURUM AND COMMON WHEAT. 17.025

FORAGE

CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002

FOREST-GENETICS

CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. 06.016

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018, 12.048*, 14.079*

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. 06.007

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEEO ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. 06.013

FOREST-INSECTS

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. **06.015**

FOREST-STANDS

GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND OISEASES. **06.003**

FOREST-TREES

CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. 06.016 GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

FORESTRY

CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. 06.016

DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS. 06.008

GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014

GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND OISEASES. **06.003**

GENETIC STRUCTURE AND ADAPTATION OF NATURAL

POPULATIONS OF SPRUCE. **06.007**INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE AND JACK PINES. 06.004

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN

SOUTHERN OREGON. 06.013 GENES FROM PHANEROCHAETE CHRYSOSPORIUM. FRAGMENT 06.012, 66.011* GENETIC MAPPING OF PEAS, LENTILS AND GENETICS OF FUNGAL PLANT PATHOGENS. 67.051 CHICKPEAS. 12.042 VARIATION IN LETTUCE DOWNY MILOEW. 12.008 FRAGMENT-LENGTH-POLYMORPHISMS FUNGUS-PHYSIOLOGY BIOCHEMISTRY OF PLANT CUTICLE. 67.108 RESEARCH ON THE INTRASPECIFIC VARIATION IN CENOCOCCUM GEOPHILUM FR.. 67.076 FUSARIUM FRAGMENT-POLYMORPHISM GENETICS AND BREEDING OF COOL SEASON CROPS. EVALUATION OF GENETIC VARIABILITY IN 12.011 ECHINACEA. 25.005, 28.003* GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, FRAGMENT-POLYMORPHISMS 15.004*. 17.018* FUSARIUM-GRAMINEARUM RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN SORGHUM. 15.009 GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*. 17.018* FRAGMENTS GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006 FUSARIUM-MONILIFORME VARIATION IN LETTUCE OOWNY MILOEW. 12.008 GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, FREE-RADICALS 15.004*, 17.018* SYSTEMS FOR THE MOLECULAR ANALYSIS OF FUSARIUM-OXYSPORUM BREEDING, DISEASES AND CULTURE OF DRY PEAS OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062 ANO LENTILS. 12.040, 62.006* FREEZING-RESISTANCE FUSARIUM-ROSEUM CELLULAR AND MOLECULAR GENETICS FOR CROP GENETICS AND PHYSIOLOGY OF FUSARIA. 14,034. IMPROVEMENT. 67.027 15.004*, 17.018* FUSARIUM-SOLANI BIOCHEMISTRY OF PLANT CUTICLE. 67.108 CELLULASE GENE EXPRESSION OURING FRUIT BREEDING, DISEASES AND CULTURE OF ORY PEAS OEVELOPMENT. 67.004 CHILLING AND GENE EXPRESSION IN FRUIT. 12.006 ANO LENTILS. 12.040, 62.006* MOLECULAR GENETIC APPROACHES FOR GERMPLASM DEVELOPMENT OF FRUIT AND NUT CROPS. 10.001 EXPRESSION OF MAIZE MITOCHONORIAL GENOME. REGULATION OF ETHYLENE INDUCED GENE EXPRESSION OURING FRUIT RIPENING. 67.009 FRUIT-DEVELOPMENT CELLULASE GENE EXPRESSION OURING FRUIT OEVELOPMENT. 67.004 GALACTOSIDASES FRUIT-PROCESSING ISOLATION AND GENETICS OF GENES INVOLVED IN REGULATION OF ETHYLENE INDUCED GENE EXOPOLYSACCHARIOE BIOSYNTHESIS OF EXPRESSION OURING FRUIT RIPENING. 67.009 RHIZOBIUM JAPONICUM. 23.027 FRUIT-QUALITY MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE REGULATION OF ETHYLENE INDUCED GENE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. EXPRESSION OURING FRUIT RIPENING. 67.009 23.002, 66.001* FRUIT-TREES GAMETES PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL GENETIC CONTROL OF SEMIGAMY; AND OERIVATION CONTROL OF XANTHOMONAO DISEASES OF FRUIT OF NULLISOMIC COTTON, 21.013 TREES. 09.006, 10.004*, 66.005* GEL-ELECTROPHORESIS FUNGAL-GENETICS CELL SPECIFIC GENE EXPRESSION OURING MOLECULAR BIOLOGY OF CELLULOSE AND LIGNIN LATICIFER OIFFERENTIATION IN POPPY. 67.096 OEGRADATION BY PHANEROCHAETE INHERITANCE OF MITOCHONORIAL ONA IN CHRYSOSPORIUM. 06.017 SOMACLONAL VARIANTS. 20.003 MAPPING THE SYMBIOSIS GENES OF PEA. 67.077 BIOCHEMISTRY OF PLANT CUTICLE. 67.108 MOLECULAR AND GENETIC BASIS OF PATHOGENESIS ECOLOGY AND BIOCONTROL OF SOILBORNE OF PSEUDOMONAS SPECIES. 67.034 PATHOGENS. 13.003, 21.010* **GENE** EXPLOITATION OF RIBOSOMAL ONA TO CONTROL CELL WALLS OF MAIZE PERICARP. 15.010 PLANT PATHOGENIC FUNGI. 67.101 EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS GENETICS AND PHYSIOLOGY OF FUSARIA. 14,034. 15.004*, 17.018* COLLECTIO. 17.008, 18.011* GENETICS OF ASPERGILLUS FLAVUS. 67.036 GENE-ACTION **FUNGUS** CHARACTERIZATION OF NITROGEN FIXATION GENES RESEARCH ON THE INTRASPECIFIC VARIATION IN ANO THEIR PRODUCTS FROM AZOTOBACTER CENOCOCCUM GEOPHILUM FR.. 67.076 VINELANOII. 23.057 FUNGUS-DISEASES-(PLANTS) CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS BIOCHEMISTRY OF PLANT CUTICLE. 67.108 AND CYTOGENETICS. 14.043 ECOLOGY AND BIOCONTROL OF SOILBORNE GENETIC CONTROL OF SEMIGAMY AND OERIVATION OF PATHOGENS. 13.003, 21.010* NULLISOMIC COTTON. 21.014, 21.015 GENETICS AND BREEDING OF COOL SEASON CROPS. GENETIC CONTROL OF SEMIGAMY; AND OERIVATION 12.011 OF NULLISOMIC COTTON. 21.013 GENETICS OF ASPERGILLUS FLAVUS. 67.036 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH GENETICS OF FUNGAL PLANT PATHOGENS. 67.051 AND PRODUCTION EFFICIENCY. 67.104 PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF GENETICS AND IMPROVEMENT OF CORN USING TROPICAL FRUIT AND THEIR CONTROL. 09.004 MOLECULAR AND TRADITIONAL METHODS. 14.057 SOYBEAN BROWN STEM ROT. 23.008 MECHANISMS OF PATHOGENESIS AND RESISTANCE IN VARIATION IN LETTUCE OOWNY MILOEW. 12.008 PLANT-PARASITE INTERACTIONS. 18.004, FUNGUS-GENETICS 23.005* CLONING AND CHARACTERIZATION OF LIGNINASE ORGANIZATION AND CONTROL OF THE HYDROLASE

```
GENES IN BARLEY. 18.018
```

GENE-ANALYSIS

- CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF DS ELEMENT. 14.053
- CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.010
- CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006
- CONTROL OF GENE EXPRESSION IN POTATO. 11.002 CRUCIFER DISEASES. 12.047
- CYTOGENETIC MANIPULATIONS FOR ALFALFA
- IMPROVEMENT. 20.002
 GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033 GENETICS AND CYTOLOGY OF MAIZE. 14.022
- HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002
- ISOLATION AND TRANSFORMATION OF A WOUND-INDUCEO TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015
- ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080
- MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA. 67.069
- MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003
- MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067
- MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017
- MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046. 67.109*
- REGULATION OF ETHYLENE INDUCEO GENE EXPRESSION DURING FRUIT RIPENING. 67.009
- STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED OURING NITROGEN FIXATION. 67.084
- THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 GENE-CLONING
 - CELL SPECIFIC GENE EXPRESSION DURING LATICIFER DIFFERENTIATION IN POPPY. 67.096
 - CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA DNA. 06.011
 - CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*
 - CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006
 - DEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING DISEASE RESISTANCE INTO POTATOES. 11.018
 - EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007
 - FUNCTION OF PLANT RNA-DEPENDENT RNA POLYMERASES. 12.036
 - GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007,
 - GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065
 - GENETIC ENGINEERING OF OILSEED SPECIES TO IMPROVE OIL QUALITY. 23.020, 25.003*
 - GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004
 - GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*
 - GENETIC/MOLECULAR CHARACTERIZATION OF THE MUTATOR-RELATED CY TRANSPOSABLE ELEMENT

- SYSTEM OF MAIZE. 14.031
- GENETICS OF FUNGAL PLANT PATHOGENS. 67.051 ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIDE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027
- ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.029, 14.030
- ISOLATION OF GENES THAT ENCODE ONA BINDING PROTEINS. **67.066**
- ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELOIDOGYNE INCOGNITA. 67.053 ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS DEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC
- ACIO. 14.001 ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*
- MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES.
- 16.004, 17.020*, 23.031*, 67.056*
 MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA. 67.069
- MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034
- MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004
- MOLECULAR CONTROL OF GENE ACTIVITY DURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003
- MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017
- MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONADS. 12.046, 67.109*
- REGULATION OF PLANT GENE EXPRESSION BY DNA METHYLATION. 67.110
- STRUCTURE AND EXPRESSION OF PLANT GENES ENCOOING CALMODULIN. 18.014
- SYSTEMS FOR THE MOLECULAR ANALYSIS OF OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062
- THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 THE MOLECULAR BIOLOGY OF THE RICE
- ALPHA-AMYLASE GENES. 16.001 THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045
- VARIATION IN LETTUCE DOWNY MILDEW. 12.008 GENE-CODING
 - STRUCTURE AND FUNCTION OF SELF-INCOMPATIBILITY GENES: A BIOTECHNOLOGICAL APPROACH. 11.013, 67.090*

GENE-DEFICIENCY

ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS OEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACID. 14.001

GENE-DOSAGE

GENETIC REGULATION OF A SEEO-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*
GENE-ENVIRONMENT-INTERACTION

- ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003
- CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048
- DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004
- OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015
- ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003
- ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055
- REGULATION OF ETHYLENE INDUCED GENE

EXPRESSION OURING FRUIT RIPENING. **67.009**STRUCTURE AND EXPRESSION OF SOYBEAN LEAF
STORAGE PROTEIN GENES. **23.043**

GENE-EXPRESSION

- ANALYSIS OF SEEO GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016
 BIOCHEMISTRY OF PLANT CUTICLE. 67.108
 CELL SPECIFIC GENE EXPRESSION OURING
- LATICIFER OIFFERENTIATION IN POPPY. 67.096
 CELLULAR AND MOLECULAR GENETICS FOR CROP
 IMPROVEMENT 18 010 63.002 66.002*
- IMPROVEMENT. 18.010, 63.002, 66.002*,
 67.026*, 67.027, 70.001*
 CELLULAR REGULATION OF THE EXPRESSION OF
- ORMANT GENES IN CEREAL GRAINS. 62.002 CELLULASE GENE EXPRESSION OURING FRUIT
- CELLULASE GENE EXPRESSION OURING FRUIT OEVELOPMENT. **67.004**
- CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007
- CHARACTERIZATION OF PLANT GENES ENCOOING
 PHOTOSYNTHETIC MEMBRANE PROTEINS. 67.074
- CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013
- CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048
- CHILLING AND GENE EXPRESSION IN FRUIT. 12.006 CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
- CHROMUSUME SEGMENTAL EFFECTS IN CURN. 14.046
 CLONING ANO CHARACTERIZATION OF LIGNINASE
 GENES FROM PHANEROCHAETE CHRYSOSPORIUM.
 06.012. 66.011*
- CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006
- CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING OIHYOROFOLATE REDUCTASE. 23.053
- CONTROL OF GENE EXPRESSION IN POTATO. 11.002
 DEVELOPMENT AND USE OF NEAR ISOGENIC LINES
 FOR GENETIC ANALYSIS OF YIELO AND GENE
 EXPRESSION. 67.018
- OEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004
- OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015
- ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEDLING. 14.070
- EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*
- EXPRESSION OF MAIZE MITOCHONORIAL GENOME.
 14.002
- EXPRESSION OF MELANIN IN PLANTS MONITORING GENE EXPRESSION. 12.002, 13.001, 67.001
- EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007
- FUNCTION OF PLANT RNA-OEPENOENT RNA POLYMERASES. 12.036
- GENE EXPRESSION IN PLANT OEVELOPMENT. 23.033
 GENE EXPRESSION IN PLANTS: A STUDY OF
- POLYADENYLATION IN PLANTS. 20.007
 GENE REGULATION, EXPRESSION, AND MOLECULAR
- GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007, 15.001*
- GENE STRUCTURE AND EXPRESSION IN PLANTS.
 14.027
- GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005
- GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE.
- GENETIC ENGINEERING OF OILSEED SPECIES TO IMPROVE OIL QUALITY. 23.020, 25.003*
 GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM,
- GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM THE SOYBEAN SYMBIONT, FOR IMPROVEO

- AGRONOMIC PROPERTIES. 23.039
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 67.104
- GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018
- HYBRIO VARIEGATION IN PHASEOLUS VULGARIS. 23.038
- IMPROVING THE EFFICACY OF BACULOVIRUS
 PESTICIOES BY RECOMBINANT ONA TECHNOLOGY.
 65.002, 67.031*
- ISOLATION AND CHARACTERIZATION OF CORN GENES
 TO ELUCIDATE GENE FUNCTION AND REGULATION.
- ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003
- ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027
- ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015
- ISOLATION OF GENES THAT ENCODE ONA BINOING PROTEINS. 67.066
- ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*
- ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058
- MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH ANO OEVELOPMENT. **67.015**
- MECHANISMS OIRECTING HORMONAL AND OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026
- MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005
- MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069
- MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*
- MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011
- MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003
- MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022
- MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. 67.089
- ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. **65.004**, **67.040***
- ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. **62.001**
- ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. 67.092
- ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055
- PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081, 67.082
- PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. **67.041**
- PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028 PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001, 21.016
- RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*
- REGULATION OF ETHYLENE INDUCEO GENE EXPRESSION OURING FRUIT RIPENING. 67.009

- REGULATION OF FATTY ACID SYNTHESIS PROTEINS IN SDYBEAN. 23.037
- REGULATION DF GENE EXPRESSIDN BY ABSCISIC ACID DURING DRDUGHT STRESS. 67.016
- REGULATION DF GENE EXPRESSIDN IN PLANTS.
 14.020
- REGULATION OF PLANT GENE EXPRESSION BY CELL TURGOR OR ABSCISIC ACID. 67.093
- REGULATION OF PLANT GENE EXPRESSION BY DNA METHYLATION. **67.110**
- REGULATION DF SDYBEAN PROTEIN GENE EXPRESSION. 23.003
- REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSION. 23.001
- REGULATORY MECHANISMS IN THE BIDSYNTHESIS DF ARDMATIC CDMPDUNDS IN HIGHER PLANTS. 67.045
- REVERSE TRANSCRIPTASE AND REPLICATION DF CAULIFLDWER MDSAIC VIRUS. 67.061
- SDYBEAN STDRAGE PROTEINS: BIDCHEMISTRY AND GENETICS. 23.013
- STRUCTURE AND EXPRESSION OF PLANT GENES ENCOOING CALMODULIN. 18.014
- STRUCTURE AND EXPRESSION OF SDYBEAN LEAF STORAGE PROTEIN GENES. 23.043 STRUCTURE, EXPRESSIDN AND EVOLUTION OF
- STRUCTURE, EXPRESSIDN AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED DURING NITROGEN FIXATION. 67.084
- SYSTEMS FDR THE MOLECULAR ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062
- THE GLUTATHIONE S-TRANSFERASE GENES FROM CDRN AND CDTTDN. 14.068, 21.007*
- THE PHYTOCHRDME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION. 18.036, 63.007*
- THE ROLE DF POLYAMINES OURING STRESS-INDUCEO ALTERATIONS IN GENE EXPRESSION. 67.073
- TISSUE-SPECIFIC GENE REGULATION IN MAIZE. 14.050
- TDWAROS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017
- TUBULIN GENES OF PLANTS. 67.060
- VIRAL GENE EXPRESSION IN POTYVIRUS INFECTEO CELLS. **67.054**
- WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

GENE-FLOW

GENETICS DF BIOTYPES IN THE HESSIAN FLY (MAYETIDLA OESTRUCTDR). 17.014

GENE-FLOWS

PDPULATION STRUCTURE AND THE EVDLUTION DF RESISTANCE IN HELIDTHIS VIRESCENS. 21.004

GENE-FUNCTION

- CLDNING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*
- ISDLATION ANO CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059
- MECHANISM DF ACTIDN OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH ANO DEVELOPMENT. **67.015**
- PLASMIDS IN PLANT PATHOGENIC BACTERIA. 18.012, 26.001*
- REGULATION DE TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*

GENE-INTERACTION

- GENETIC AND CYTOGENETIC ANALYSIS DF AGRDNOMIC AND QUALITY CHARACTERISTICS DF DURUM AND CDMMDN WHEAT. 17.025
- GENETICS AND CYTDLDGY OF MAIZE. 14.022
 GENOME ORGANIZATION IN THE CULTIVATED AND WILD SPECIES OF TOMATO. 12.015
- REGULATION OF SOYBEAN SEED PROTEIN GENE

EXPRESSIDN. 23.001

GENE-ISOLATION

- TRANSPOSDN MUTAGENESIS IN TDMATD. 12.016 GENE-LOCI
 - BACTERIAL GENES CDDING FOR PLANT RIBULDSE BISPHOSPHATE CARBDXYLASE/DXYGENASE. **67.098**
 - CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012
 - CHARACTERIZATIDN DF NITRDGEN FIXATIDN GENES AND THEIR PRODUCTS FROM AZDTDBACTER VINELANDII. 23.057
 - CHARACTERIZATION DF THE NATURAL VARIABILITY IN PINUS RADIATA DNA. **06.011**
 - CHRDMATIN STRUCTURE AND GENE EXPRESSIDN IN MAIZE. 14.008
 - CHRDMDSDMAL LOCATIONS AND EVOLUTION DF HOMDLDGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*
 CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT
 - CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIOIZE TO UNIQUE-SEQUENCE-DNA CLONES. 17.031
 - CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
 CYTOGENETIC ANALYSIS DF RESTRICTION FRAGMENT
 LENGTH POLYMORPHISMS (RFLPS). 14.015
 - OEVELOPMENT ANO USE OF NEAR ISDGENIC LINES FOR GENETIC ANALYSIS OF YIELO AND GENE EXPRESSION. 67.018
 - DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056
 - ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL
 AND CHICKPEA USING ISOZYME AND DNA
 MARKERS 12 039
 - MARKERS. 12.039
 FUNCTION OF PLANT RNA-DEPENDENT RNA
 POLYMERASES. 12.036
 - GENE MAPPING IN SOYBEAN WITH MDLECULAR MARKERS. 23.034
 GENE SYNTENY RELATIONSHIPS AND MAP LDCATIONS
 - GENE SYNTENY RELATIONSHIPS AND MAP LDCATIONS IN HEXAPLOIO WHEAT AND ITS RELATIVES. 67.097
 - GENETIC CONTROL OF SEMIGAMY AND DERIVATION DF NULLISOMIC COTTDN. 21.014, 21.015
 - GENETIC STRUCTURE DF WEEO-CROP POPULATION SYSTEMS (CUCURBITA AND CHENDPODIUM).
 14.073
 - GENETICS ANO IMPROVEMENT DF CDRN USING MOLECULAR AND TRAOITIDNAL METHDOS. 14.057
 - GENETICS OF ASPERGILLUS FLAVUS. 67.036
 GENETICS OF FUNGAL PLANT PATHDGENS. 67.051
 - GENETICS OF FUNGAL PLANT PATHDGENS. **67.051**HYBRID VARIEGATION IN PHASEDLUS VULGARIS. **23.038**
 - ISDLATION OF THE RESISTANCE GENE IN TDMATD TO THE NEMATODE MELOIOOGYNE INCDGNITA. 67.053
 - ISOZYME LOCI AS MARKERS FOR LDCATING AND MANIPULATING QUANTITATIVE TRAIT LDCI. 14.058, 14.063
 - MAPPING THE SYMBIDSIS GENES OF PEA. **67.077**MOLECULAR ANALYSIS DF S-LDCUS EXPRESSIDN IN
 ANTHERS OF BRASSICA. **67.069**
 - MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011
 - MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004
 - MOLECULAR GENETIC ANALYSIS DF THE BRASSICA S LOCUS. 67.067
 - ORGANIZATION AND MANIPULATION DF WHEAT STORAGE PROTEIN GENES. 17.013
 - DRGANIZATION OF THE R CHRDMOSDME REGION IN MAIZE. 14.077
 - REGULATION OF PLANT GENE EXPRESSIDN BY DNA METHYLATION. **67.110**
 - RELATION OF ISOZYME LOCI TO QUALITATIVE
 - CHARACTERS OF SDYBEAN. 23.017
 THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007
 WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS.

17.006, 67.021*

GENE-MANIPULATION

- CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002
- OFFINING AND MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*
- OEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002
- DEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. 67.024
- EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007
- GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033 GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005
- GENOME ORGANIZATION IN THE CULTIVATED AND WILO SPECIES OF TOMATO. 12.015
- ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027
- ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058
- MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 GENE-MAPPING
 - A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103
 - ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. 67.043
 - BACTERIAL GENES COOING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098
 - CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*
 - CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012
 - CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
 - CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014
 - CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015
 - CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. 06.016
 - OEFINING AND MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*
 - OEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. 67.024
 - OEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004
 - ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. **06.015**
 - ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL ANO CHICKPEA USING ISOZYME ANO ONA MARKERS. 12.039, 12.041
 - GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY RECIPROCAL CHROMOSOME SUBSTITUTIONS. 17.024
 - GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003*
 - GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034, 23.056
 - GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007. 15.001*
 - GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOIO WHEAT AND ITS RELATIVES. 67.097
 - GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006 GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.015
 - GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015 GENETICS OF ASPERGILLUS FLAVUS. 67.036 GENETICS OF FUNGAL PLANT PATHOGENS. 67.051

- GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016
- HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002
- INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE AND JACK PINES. 06.004
- ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015
- ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELOIOOGYNE INCOGNITA. 67.053
- ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*
- MAPPING THE SYMBIOSIS GENES OF PEA. 67.077 MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*
- MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA. 67.069
- MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034
- MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011
- MOLECULAR MAPPING OF GENES IN CORN. 14.040 MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020
- MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047
- ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. 65.004, 67.040*
- PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.082
- PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016
- RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*
 REGULATION OF PLANT GENE EXPRESSION BY ONA
- METHYLATION. 67.110
- REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003
- REPEATED ONA SEQUENCES AND CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008
- STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE. 23.035
- STRUCTURE AND EXPRESSION OF PLANT GENES ENCOOING CALMOOULIN. 18.014
- STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED OURING NITROGEN FIXATION. 67.084
- THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001
- THE PHYCOCYANIN GENES OF AGMENELLUM QUAORUPLICATUM. 67.088
- THE REPLICATION OF CHLOROPLAST ONA IN CHLAMYOOMONAS REINHAROII. 67.057
- USE OF MOLECULAR MARKERS IN PLANT BREEDING ANO GENETICS. 67.071

GENE-PRODUCTS

- CELL SPECIFIC GENE EXPRESSION OURING LATICIFER OIFFERENTIATION IN POPPY. 67.096
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010
- ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEOLING. 14.070
- ISOLATION OF GENES THAT ENCODE ONA BINDING PROTEINS. **67.066**MECHANISM OF ACTION OF PLANT GROWTH
- SUBSTANCES IN PLANT GROWTH AND

OEVELOPMENT. 67.015

MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*

MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. 67.089

TOWARDS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

GENE-RECOMBINATION

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005

STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE. 23.035 TISSUE CULTURE AND THE TRANSFER OF

CYTOPLASMIC GENES. 17.027, 18.031*

GENE-REGULATION

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF DAT STORAGE PROTEINS. 18.015

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010, 67.027

CELLULASE GENE EXPRESSION OURING FRUIT OEVELOPMENT. 67.004

CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF OS ELEMENT. 14.053

CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*

COOING AND REGULATION OF MITOCHONDRIAL GENES. 14.052

DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004

EFFICIENCY OF NITROGEN FIXATION. 23.025,

GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007, 15.001*

GENETIC MECHANISMS IN CORN. 14.044 GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*,

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION ANO FUNCTION. 14.059

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005

MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA. 67.069

MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067

MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022

MOLECULAR MANIPULATION OF GENES. 28.002 PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.082

PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075

REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037

REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSION. 23.001

STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREOII, A NEWLY OESCRIBEO SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED OURING NITROGEN FIXATION. 67.084

SYSTEMS FOR THE MOLECULAR ANALYSIS OF

OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

TISSUE-SPECIFIC GENE REGULATION IN MAIZE. 14.050

GENE-REGULATION,

REGULATION OF GRASSHOPPER REPRODUCTION BY JUVENILE HORMONE. 67.023
REGULATION OF PLANT GENE EXPRESSION. 67.106

GENE-RX

MOLECULAR GENETICS OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.005

GENE-STRUCTURE

ANALYSIS OF SEED GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016

CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002

CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007

CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF OS ELEMENT. 14.053

CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012

CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.010

CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*

DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003
GENOME EVOLUTION IN BRASSICA. 12.012

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE FUNCTION AND REGULATION. 14.054

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059

ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003

MITOCHONORIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. 67.030 MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN

ANTHERS OF BRASSICA. 67.069

MOLECULAR AND GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034

MOLECULAR CONTROL OF GENE ACTIVITY DURING REPRODUC TIVE OEVELOPMENT IN THE WHEAT PLANT. 17.003

MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067

MOLECULAR MANIPULATION OF GENES. 28.002 ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. **62.001**

ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055

ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013

PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028 PRESERVATION AND UTILIZATION OF GERMPLASM IN

COTTON. 21.001 REGULATION OF GENE EXPRESSION BY ABSCISIC ACID OURING OROUGHT STRESS. 67.016

REGULATION OF GENE EXPRESSION IN PLANTS. 14.020

STRUCTURAL ANALYSIS, OISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE. 23.035 STRUCTURE AND EXPRESSION OF PLANT GENES ENCODING CALMOOULIN. 18.014 STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043 STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREOII, A NEWLY DESCRIBEO SPECIES OF SOYBEAN RHIZ. 23.051, 66.009* STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS IN MAIZE. 14.072 STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006* THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION ANO EXPRESSION. 18.036, 63.007* TUBULIN GENES OF PLANTS. 67.060 WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006. 67.021* GENE-TAGGING GENETIC ANALYSES OF THE MUTATOR SYSTEM OF MAIZE. 14.028 GENE-TRANSFER CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.028 CELLULASE GENE EXPRESSION OURING FRUIT OEVELOPMENT. 67.004 CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006 CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. 06.016 OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING OISEASE RESISTANCE INTO

1

- OEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. 67.024 POTATOES. 11.018 EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032 GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033 GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003* GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM. THE SOYBEAN SYMBIONT, FOR IMPROVEO AGRONOMIC PROPERTIES. 23.039
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH ANO PRODUCTION EFFICIENCY. 12.010, 67.104 GENETIC IMPROVEMENT OF PROPIONIBACTERIA ANO OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001* GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003 ISOLATION OF GENES IMPLICATED IN THE CONTROL
- OF IMPORTANT AGRONOMIC CHARACTERS. 14.017. 23.010* MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES.
- 16.004, 17.020*, 23.031*, 67.056* MOLECULAR GENETIC APPROACHES FOR GERMPLASM OEVELOPMENT OF FRUIT AND NUT CROPS. 10.001
- PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016
- REGULATION OF SOYBEAN SEEO PROTEIN GENE EXPRESSION. 23.001
- TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031* TISSUE CULTURE GENETIC SYSTEMS. 14.041,
- 18.022* WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*
- GENE-TRANSFER.
- TRANSPOSON MUTAGENESIS IN TOMATO. 12.016
- A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIDIUM PASTEURIANUM AND OTHER

- MICROORGANISMS. 67.103
- BACTERIAL GENES COOING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 18.010, 18.013, 23.006*, 24.001*, 26.002*, 63.002, 66.002*, 67.026*, 67.027, 67.028, 70.001*
- CELLULASE GENE EXPRESSION OURING FRUIT DEVELOPMENT. 67.004
- CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012
- CHARACTERIZATION OF PLANT GENES ENCODING PHOTOSYNTHETIC MEMBRANE PROTEINS. 67.074
- CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013
- CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES COOING FOR SPECIFIC GENES. 67.038
- CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. 06.011
- CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048
- CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.010
- CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIOIZE TO UNIQUE-SEQUENCE-DNA CLONES. 17.031
- CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. 67.014
- CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006
- CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIOS AND CYBRIOS OF TOMATO. 12.030
- CONTROL OF GENE EXPRESSION IN POTATO. 11.002 CONTROL OF THE BIOSYNTHESIS OF PROTEINS & AMINO AC IOS IN LEGUME SEEDS. 23.046
- OFFINING AND MAPPING THE GENES OF
- CAULIMOVIRUSES. 66.004, 67.055* OEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002
- OEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. 14.061
- OEVELOPMENTAL GENETICS USING THE ALCHOL
- OEHYOROGENASE GENE-SYSTEM IN MAIZE. 14.004 OEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004
- **OISSECTION OF HETEROSIS OF QUANTITATIVE** TRAITS USING MOLECULAR MARKERS. 14.056
- ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE **SEEOLING. 14.070**
- EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*
- EXPRESSION OF MAIZE MITOCHONORIAL GENOME. 14.002
- FUNCTION OF PLANT RNA-OEPENOENT RNA POLYMERASES. 12.036
- GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034
- GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027
- GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC ANO QUALITY CHARACTERISTICS OF OURUM ANO COMMON WHEAT. 17.025
- GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVEO AGRONOMIC PROPERTIES. 23.039
- GENETICS AND CYTOLOGY OF MAIZE. 14.022
- GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANOII. 23.048, 23.049, 66.006*, 66.007*
- GENETICS, CYTOGENITCS OF COTTON GERMPLASM.

21 008

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*

GENOME ORGANIZATION IN THE CULTIVATED AND WILO SPECIES OF TOMATO. 12.015

GLIADIN GENES OF COMMON WHEAT AND ITS

ANCESTORS. 17.011
HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002

HYBRIO VARIEGATION IN PHASEOLUS VULGARIS. 23.038

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE STRUCTURE, REGULATION ANO FUNCTION. 14.059

ISOLATION AND CHARACTERIZATION OF GENES REGULATEO BY LIGHT. 67.080

ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003
ISOLATION AND GENETICS OF GENES INVOLVED IN

EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017. 23.010*

ISOLATION OF GENES THAT ENCODE ONA BINDING PROTEINS. 67.066

ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELOIOOGYNE INCOGNITA. 67.053

ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS OEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN **SOYBEANS. 23.012**

MAPPING THE SYMBIOSIS GENES OF PEA. 67.077 MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006

MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE, 14.011

MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067

MOLECULAR GENETIC APPROACHES FOR GERMPLASM DEVELOPMENT OF FRUIT AND NUT CROPS. 10.001

MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA OUCH.. 10.005

MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. **23.022**

MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*

MOLECULAR MANIPULATION OF GENES. 28.002 MOLECULAR SWITCHES IN PLASTIO

OIFFERENTIATION. 67.089

MUTANT GENES THAT AFFECT ENOOSPERM OEVELOPMENT IN MAIZE. 14.078

ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. 62.001

ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055

ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013

ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077

PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081, 67.082

PHYSICAL MAPPING OF THE GENOME OF WHEAT.

17.016

PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075

RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*

REGULATION OF ETHYLENE INDUCED GENE EXPRESSION OURING FRUIT RIPENING. 67.009

REGULATION OF SOYBEAN PROTEIN GENE

EXPRESSION. 23.003
REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*

REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNOS IN HIGHER PLANTS. 67.045

STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE. 23.035

STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043

STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREOII, A NEWLY OESCRIBED SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

STRUCTURE OF GENES INVOLVED IN DIL SYNTHESIS IN MAIZE. 14.072

STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE & THEIR USE AS RESEARCH TOOLS. 14.018

SYSTEMS FOR THE MOLECULAR ANALYSIS OF OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN ANO COTTON. 14.068, 21.007*
THE MOLECULAR BIOLOGY OF THE RICE

ALPHA-AMYLASE GENES. 16.001

THE PHYCOCYANIN GENES OF AGMENELLUM QUAORUPLICATUM. 67.088

THE ROLE OF POLYAMINES OURING STRESS-INDUCED ALTERATIONS IN GENE EXPRESSION. 67.073

GENETIC

CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE.

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

GENETIC-CODE

COOING AND REGULATION OF MITOCHONORIAL GENES. 14.052

GENETIC-COMPATIBILITY

GENETICS OF ASPERGILLUS FLAVUS. 67.036 MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. **67.067**

GENETIC-CORRELATION

MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA OUCH... 10.005

GENETIC-DIVERSITY

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL ANO CHICKPEA USING ISOZYME ANO ONA MARKERS. 12.039

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIDIZING POPULATIONS. 14.051

GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS. 14.057

ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA ANO OTHER EUROPEAN COUNTRIES. 14.080
MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC

MALE STERILE AND FERTILE PLANTS. 62.005

MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017

MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020

MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021

- PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001
- RECOMBINANT DNA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*
- THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045

GENETIC-ENGINEERING

- A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103
- ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. 67.043
- CELLULAR ANO MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*, 67.028, 67.035, 70.001*
- CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012
- CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL ANO MUTANT ZEA MAYS. 67.014
- CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006
- CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING OIHYOROFOLATE REDUCTASE. 23 053
- CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIOS AND CYBRIOS OF TOMATO. 12.030
- OEFINING ANO MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*
- OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING OISEASE RESISTANCE INTO POTATOES. 11.018
- DEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. 14.061
- EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*
- GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003*
- GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027
- GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006
 GENETIC ENGINEERING OF OILSEED SPECIES TO
 IMPROVE OIL QUALITY. 23.020, 25.003*
- GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVEO AGRONOMIC PROPERTIES. 23.039
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018, 07.001, 12.010, 12.048*, 14.079*, 17.023*, 18.029*, 67.104
- GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOCOS. 14.025, 31.001*
- GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*, 21.005*, 23.040*
- GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPOOIUM). 14.073
- GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANOII. 23.048, 66.006*
- GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008
- GENOME ORGANIZATION IN THE CULTIVATEO AND WILO SPECIES OF TOMATO. 12.015
- GLYPHOSATE OEGRAOATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*
- INHERITANCE OF MITOCHONORIAL ONA IN SOMACLONAL VARIANTS. 20.003
- ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM

- ALFALFA. 20.015
- ISOLATION OF A LIGNIN-BIOSYNTHESIS GENE FROM LOBLOLLY PINE. **06.010**
- ISOLATION OF GENES IMPLICATEO IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017. 23.010*
- ISOLATION OF GENES THAT ENCODE ONA BINOING PROTEINS. 67.066
- ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATOOE MELOIOOGYNE INCOGNITA. 67.053
- MOLECULAR GENETIC APPROACHES FOR GERMPLASM OEVELOPMENT OF FRUIT AND NUT CROPS. 10.001
- MOLECULAR GENETICS OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.005
- MOLECULAR MANIPULATION OF GENES. 28.002
- ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. **65.004**, **67.040***
- PLASMIDS IN PLANT PATHOGENIC BACTERIA. 18.012, 26.001*
- RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*
- REGULATION OF GENE EXPRESSION IN PLANTS. 14.020
- REGULATION OF PLANT GENE EXPRESSION BY ONA METHYLATION. 67.110
- THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001
- TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001
- USE OF MOLECULAR MARKERS IN PLANT BREEDING AND GENETICS. 67.071
- VIRAL GENE EXPRESSION IN POTYVIRUS INFECTEO CELLS. 67.054
- WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

GENETIC-MAPPING

- ANEUPLOIO ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.013, 26.002*
- CHROMOSOMAL LOCALIZATION AND INTRACHROMOSOMAL MAPPING OF WHEAT RFLP LOCI. 17.032
- CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*
- CONSTRUCTION OF A SATURATEO GENETIC LINKAGE MAP FOR LOBLOLLY PINE. **06.002**
- COSMIO MAPPING OF MITOCHONORIAL ONA OF MALE FERTILE AND MALE STERILE SORGHUM. 15.002
- CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015
- DEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002
- OEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. 67.024
- ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039
- FURTHER OEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034
- GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034
- GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027
- GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF OURUM AND COMMON WHEAT. 17.025
- COMMON WHEAT. 17.025
 GENETIC CONTROL OF LEAF LESION FORMATION IN MAIZE. 14.047
- GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH

- ANO PRODUCTION EFFICIENCY. 67.104 GENETIC MANIPULATION OF LACTIC DEHYOROGENASE
- ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019
- GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*, 21.005*, 23.040*
- GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018
- ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027
- ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080
- MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*
 MECHANISMS DIRECTING HORMONAL AND
- OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026
- MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017
- ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017
- PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016
- PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, DOCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001
- PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.016
- TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001
- USE OF SINGLE COPY CLONEO ONA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028 VARIATION IN LETTUCE DOWNY MILDEW. 12.008
- GENETIC-MAPS RFLP AND MOLECULAR ANALYSIS OF ROOT KNOT NEMATODES, NEMATODE INFECTED PLANTS AND

PEACHES. 10.009, 23.054* GENETIC-MARKERS

- CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF DS ELEMENT. **14.053**CLASSICAL GENE MAPPING IN SOYBEAN USING
- MOLECULAR MARKERS. 23.014
- OEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELO AND GENE EXPRESSION. 67.018
- OEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002
- DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056
- EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIDIZING POPULATIONS. 14.051
- EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032
- FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034
- GENE MAPPING IN SOYBEAN WITH MOLECULAR
- MARKERS. 23.034, 23.056
 GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065
- GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011
- GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS. 14.057
- GENETICS, CYTOGENITCS OF COTTON GERMPLASM.
- GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

- GENOME EVOLUTION IN BRASSICA. 12.012 MODULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021
- MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017
- MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020
- MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021
- ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077
- PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016
- PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, ANO DISTRIBUTION. 25.001
- PRESERVATION AND UTILIZATION OF GERMPLASM IN
- COTTON. 21.016 Structure, Variation and Inheritance of RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036
- STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE & THEIR USE AS RESEARCH TOOLS. 14.018 TOWARDS A TRANSPOSON TAGGING SYSTEM IN
- TOMATO. 12.017
- USE OF MOLECULAR MARKERS IN PLANT BREEDING ANO GENETICS. 67.071
- VARIATION IN LETTUCE DOWNY MILDEW. 12.008

GENETIC-RECOMBINATION

- CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002
- GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013
- IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIDES BY RECOMBINANT ONA TECHNOLOGY. 65.002, 67.031*
 ISOLATION OF GENES THAT ENCODE DNA BINOING
- PROTEINS. **67.066**
- MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034
- MOLECULAR MANIPULATION OF GENES. 28.002 ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077
- TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001
- USE OF SINGLE COPY CLONED ONA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028

GENETIC-REGULATION

- BACTERIAL GENES COOING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*, 63.002, 66.002*, 67.026*, 67.028. 70.001*
- CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002
- CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008
- CONTROL OF GENE EXPRESSION IN POTATO. 11.002 DEVELOPMENTAL GENETICS USING THE ALCHOL
- DEHYDROGENASE GENE-SYSTEM IN MAIZE. 14.004 OIFFERENTIAL EXPRESSION OF PROTEINASE
- INHIBITOR GENES IN PLANT TISSUES. 11.015 GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE.
- 14.065 GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*,
- 67.063* GENETICS AND CYTOLOGY OF MAIZE. 14.022 GENETICS OF NITRATE REDUCTION IN BARLEY.
- 67.105 MOLECULAR CHARACTERIZATION OF THE SUCROSE

SYNTHETASE-2 GENE OF MAIZE. 14.011 REGULATION OF ETHYLENE INDUCED GENE EXPRESSION OURING FRUIT RIPENING. 67.009 REGULATION OF GENE EXPRESSION IN PLANTS. REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003 REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSION. 23.001 STRUCTURE AND EXPRESSION OF PLANT GENES ENCODING CALMODULIN. 18.014 STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043 STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREOII, A NEWLY DESCRIBEO SPECIES OF SOYBEAN RHIZ. 23.051, 66.009* THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION. 18.036, 63.007* TUBULIN GENES OF PLANTS. 67.060 GENETIC-RESPONSE MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND OEVELOPMENT. 67.015 PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001 GENETIC-STOCKS CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF OS ELEMENT. 14.053 GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006 GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014 GENOME EVOLUTION IN BRASSICA. 12.012 MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060 GENETIC-STOCKSCRYOPRESERVATION BARLEY GENETICS AND PLANT CYTOGENETICS. 18.008, 20.004*, 27.001*, 67.022* GENETIC-TRANSFERS DEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELO AND GENE EXPRESSION. 67.018 GENETIC MECHANISMS IN CORN. 14.044 GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018 ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010* TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001 18,003, 67,006* BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014 CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. 06.011 CRUCIFER DISEASES. 12.047

GENETIC-VARIANCE

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIDIZING POPULATIONS. 14.051

GENETIC MECHANISMS IN CORN. 14.044

GENETIC STRUCTURE OF CULTIVATED SOYBEANS AND THE WILO SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045

GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPOOIUM).

MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEEO ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. 06.013

TISSUE CULTURE GENETIC SYSTEMS. 14.041,

18.022*

GENETICS

18.003, 67.006*

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103

A STUDY OF GENETIC DIVERSITY IN PEANUTS USING CHLOROPLAST AND RRNA MOLECULAR MARKERS. 24.002

BIOCHEMICAL AND DEVELOPMENTAL GENETICS OF HIGHER PLANTS. 14.038, 18.020

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.013, 26.002*, 63.002, 66.002*, 67.026*, 67.035, 70.001*

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008

CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS. 14.032, 23.024*,

30.001*, 31.002* DEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELO AND GENE EXPRESSION. 67.018

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. 06.015

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.041

EXPLOITATION OF RIBOSOMAL ONA TO CONTROL PLANT PATHOGENIC FUNGI. 67.101

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 12.010, 17.023*, 18.029*

GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPOOIUM). 14.073

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003

GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANDII. 23.048, 23.049, 66.006*, 66.007*

GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059

ISOLATION OF GENES THAT ENCODE DNA BINDING PROTEINS. **67.066**

MODULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006

MUTANT GENES THAT AFFECT ENOOSPERM DEVELOPMENT IN MAIZE. 14.078

ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN SORGHUM. 15.009

STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES

OF A SOYBEAN INSECTION SEQUENCE. 23.035 STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREDII, A NEWLY OESCRIBED SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

THE PHYCOCYANIN GENES OF AGMENELLUM

QUADRUPLICATUM. 67.088

THE PHYTOPATHOGENIC BACTERIA. 67.052 TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

GENOME-EVALUATION

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL

ANO CHICKPEA USING ISOZYME ANO ONA MARKERS. 12.041

GENOMES

- 18.003, 67.006*
- CELLULAR ANO MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010
- CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. **62.002**
- CELLULASE GENE EXPRESSION OURING FRUIT OEVELOPMENT. 67.004
- CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHODS. 17.007
- CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES COOING FOR SPECIFIC GENES. 67.038
- CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. 06.011
- CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIOIZE TO UNIQUE-SEQUENCE-ONA CLONES. 17.031
- CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
 CONSTRUCTION AND CHARACTERIZATION OF SOMATIC
 HYBRIOS AND CYBRIOS OF TOMATO. 12.030
- CONTROL OF THE BIOSYNTHESIS OF PROTEINS & AMINO AC IOS IN LEGUME SEEDS. 23.046
- CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043
- CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002
- OEFINING AND MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*
- OEVELOPMENT OF A ONA HYBRIOIZATION ASSAY FOR OETECTION OF THE RING ROT PATHOGEN. 11.007
- OEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS. OG. 008
- ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039
- EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*
- EXPRESSION OF MAIZE MITOCHONORIAL GENOME. 14.002
- GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033
 GENE REGULATION, EXPRESSION, AND MOLECULAR
 MAPPING IN MAIZE AND SORGHUM. 14.007,
 15.001*
- GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOIO WHEAT AND ITS RELATIVES. **67.097**
- GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005
- GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE AND FOOD VALUE. 12.021
- GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPODIUM).
 14.073
- GENETICS AND BREEDING OF COOL SEASON CROPS.
- GENETICS AND MOLECULAR BIOLOGY OF COTTON
 CYTOPLASMIC MALE STERTLITY, 21,003
- CYTOPLASMIC MALE STERILITY. 21.003
 GENOME EVOLUTION IN BRASSICA. 12.012
- GENOME ORGANIZATION IN THE CULTIVATED AND WILD SPECIES OF TOMATO. 12.015
- HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002
- INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE AND JACK PINES. **06.004**
- ISOLATION ANO CHARACTERIZATION OF GENES REGULATED BY LIGHT. **67.080**
- ISOLATION AND CHARACTERIZATION OF POTATO

- TUBERIZATION GENES. 11.003
- ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS DEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001
- MAPPING THE SYMBIOSIS GENES OF PEA. **67.077** MECHANISMS OIRECTING HORMONAL AND
 - OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026
- MITOCHONORIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRID CELLS OF GRASSES. 67.030
- MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. **62.005**
- MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036
 MOLECULAR AND GENETIC BASIS OF PATHOGENESIS
 OF PSEUOOMONAS SPECIES. 67.034
- MOLECULAR AND GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034
- MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067
- MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*
- MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017
- MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA OUCH.. 10.005
- MOLECULAR MANIPULATION OF GENES. 28.002
 MOLECULAR MARKERS FOR EVALUATION OF SWEET
 POTATO GERMPLASM DIVERSITY AND
 SYSTEMATICS. 12.020
- MOLECULAR SWITCHES IN PLASTID DIFFERENTIATION. 67.089
- ORGANELLE DNA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. **20.005**
- ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. **65.004, 67.040***
- ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. **62.001**
- ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055
- ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013
- ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017
- PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081, 67.082
- PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016
- PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075
- REGULATION OF PLANT GENE EXPRESSION BY DNA METHYLATION. **67.110**
- REPEATED ONA SEQUENCES AND CHLOROPLAST DNA INSTABILITY IN CLOVER. 20.008
- SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013
- STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE. 23.035 SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001
- THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045
- TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001
- USE OF MOLECULAR MARKERS IN PLANT BREEDING AND GENETICS. 67.071
- USE OF SINGLE COPY CLONEO ONA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028
- VARIATION IN LETTUCE DOWNY MILOEW. 12.008 VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. 67.054

GENOMIC

.) ,

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

GENOMIC-DNA-CLONES

CHROMOSOMAL LOCALIZATION AND INTRACHROMOSOMAL MAPPING OF WHEAT RFLP LOCI. 17.032

GENOTYPES

- CELLULAR GENETICS OF CITRUS SPECIES. **09.002**CHROMOSOMAL MAPPING OF GENES CONTROLLING
 TISSUE CULTURE RESPONSE IN WHEAT. **17.010**OISSECTION OF HETEROSIS OF QUANTITATIVE
- TRAITS USING MOLECULAR MARKERS. 14.056
- GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004
- GENETIC MECHANISMS IN CORN. 14.044
- ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080
- MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060
- MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA. 67.069
- MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067
- MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA OUCH..
- PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. **06.013**
- USE OF SINGLE COPY CLONED DNA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028

GERM-PLASM

18.003, 67.006*

- ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010
- CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014
- CROP IMPROVEMENT ANO GERMPLASM OEVELOPMENT THROUGH CHROMOSOMAL ANO CYTOPLASMIC MANIPULATIONS. 17.009
- ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039
- EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032
- GENE EXPRESSION IN PLANTS: A STUDY OF POLYADENYLATION IN PLANTS. 20.007
- GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.056
- GENETIC STRUCTURE OF CULTIVATEO SOYBEANS AND THE WILO SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045
- GENETIC STRUCTURE OF WEEO-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPOOIUM). 14.073
- GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011
- GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015
 GENETICS AND IMPROVEMENT OF CORN USING
- MOLECULAR AND TRADITIONAL METHODS. 14.057 GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON
- GENE MAPPING. 23.016
- GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008
- GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018
- GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001
- GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*
 INHERITANCE OF MITOCHONORIAL ONA IN

- SOMACLONAL VARIANTS. 20.003
- ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE FUNCTION AND REGULATION. 14.054
- ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*
- ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058, 14.063
- ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080
- MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017
- MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020
- PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035
- PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OCCUMENTATION, MAINTENANCE, AND DISTRIBUTION, 25.001
- PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028
- STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036 VEGETABLE GENETICS. 12.014

GERM-PLASM-BANKS

- GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008
- PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001
- PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001

GERMINATION

- OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*
- ROLE OF LOW MOLECULAR WEIGHT HEAT SHOCK PROTEINS IN PLANT OEVELOPMENT. 67.002

GERMPLASM

- A STUDY OF GENETIC DIVERSITY IN PEANUTS USING CHLOROPLAST AND RRNA MOLECULAR MARKERS. 24.002
- BARLEY GENETICS AND PLANT CYTOGENETICS.
 18.008, 20.004*, 27.001*, 67.022*
 GENETIC MAPPING OF PEAS, LENTILS AND
- GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

GIANT-CELLS

RFLP ANO MOLECULAR ANALYSIS OF ROOT KNOT NEMATODES, NEMATODE INFECTED PLANTS ANO PEACHES. 10.009, 23.054*

GIBBERELLIC-ACID

- MECHANISMS DIRECTING HORMONAL AND DEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026
- ORGANIZATION AND CONTROL OF THE HYOROLASE GENES IN BARLEY. 18.018

GIEMSA-BANDING

BARLEY GENETICS AND PLANT CYTOGENETICS. 18.008, 20.004*, 27.001*, 67.022*

GLIADINS

- CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007
- CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012
- GLIAOIN GENES OF COMMON WHEAT ANO ITS ANCESTORS. 17.011
- MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003

ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013 WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006. 67.021*

GLOBULINS

ANALYSIS OF SEEO GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016
ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

GLUTAMINE-SYNTHASE

PLANT GENE REGULATION DURING NITROGEN ASSIMILATION. 67.075

GLUTATHIONE

THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN AND COTTON. 14.068, 21.007*

GLUTATHIONE-TRANSFERASES

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATEO ATRAZINE TOLERANCE IN CORN. 14.069
MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067

GLUTEN

CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012
ORGANIZATION ANO MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013

GL YCTNE

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047

GLYCINE-SOJA

GENETIC STRUCTURE OF CULTIVATED SOYBEANS AND THE WILD SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015
MODULATION AND NITROGEN FIXATION OF PRC R.
JAPONICUM. 23.021

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047

RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN. 23.017

GLYCININ

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012

SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013

GLYCOPEPTIDES

SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013

GLYCOPROTEIN)

CELL WALLS OF MAIZE PERICARP. 15.010

GLYCOPROTEINS

MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004

GLYOXAL-OXIDASES

MOLECULAR BIOLOGY OF CELLULOSE AND LIGNIN DEGRADATION BY PHANEROCHAETE CHRYSOSPORIUM. **06.017**

GLYPHOSATE

GLYPHOSATE DEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

GOSSYPIUM

DEVELOPMENT OF A MOLECULAR CYTOGENETIC MAP OF COTTON BY IN SITU HYBRIDIZATION. 21.012
PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001, 21.016

GRAIN

CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002

GRAIN-AMARANTHS

PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, DOCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001

GRAIN-BREAKAGE

MAIZE GRAIN PROTEIN COMPOSITION AND DISTRIBUTION AS RELATED TO HARDNESS, HANDLING AND PROCESSING. 14.016

GRAIN-LEGUMES

BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006*

GRAIN-PROPERTIES

MAIZE GRAIN PROTEIN COMPOSITION AND DISTRIBUTION AS RELATED TO HARDNESS, HANOLING AND PROCESSING. 14.016

GRAPES

BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*

GRASSES

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.028

MITOCHONDRIAL DNA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRID CELLS OF GRASSES. **67.030** MOLECULAR MANIPULATION OF GENES. **28.002** TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. **17.001**

GRASSHOPPERS

REGULATION OF GRASSHOPPER REPRODUCTION BY JUVENILE HORMONE. 67.023

GRISEA

CLONING CULTIVAR SPECIFICITY GENES FROM MAGNAPORTHE GRISEA. 16.010

GROWTH-FACTORS

MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND OEVELOPMENT. **67.015**

GROWTH-RATE

CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010

GROWTH-REGULATORS

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

ISOLATION OF TRANSPOSON INOUCED MAIZE MUTANTS OFFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

GUAYULE

MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004

HANDLING

MAIZE GRAIN PROTEIN COMPOSITION AND DISTRIBUTION AS RELATED TO HARONESS, HANOLING AND PROCESSING. 14.016

HAPLOIDS

GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014, 21.015
GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013
GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011

HARDNESS

MAIZE GRAIN PROTEIN COMPOSITION AND DISTRIBUTION AS RELATED TO HARDNESS, HANDLING AND PROCESSING. 14.016

HARDWOODS

CYTOGENETIC STUDIES OF HARDWOOD AND CONIFEROUS FOREST TREES. **06.016**DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS. **06.008**

HBRIDIZATION

RFLP METHODOLOGY FOR RELATEDNESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

HEAT-RESISTANCE

CELLULAR METABOLISM IN PLANTS. 67.049

HEAT-SHOCK-PROTEIN

ROLE OF LOW MOLECULAR WEIGHT HEAT SHOCK PROTEINS IN PLANT OEVELOPMENT. 67.002

HEAT-STRESS

3 1

CELLULAR METABOLISM IN PLANTS. 67.049
OEVELOPMENTAL REGULATION OF SPECIFIC HEAT
SHOCK PROTEINS. 12.003, 63.001*

HEAT-TOLERANCE

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

HELIOTHIS-VIRESCENS

POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS. 21.004

HELIOTHIS-ZEA

GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*, 21.005*, 23.040*

HELMINTHOSPORIUM-MAYDIS

GENETICS OF FUNGAL PLANT PATHOGENS. 67.051

HERBICIDE-RESISTANCE

BIOCHEMICAL AND OEVELOPMENTAL GENETICS OF HIGHER PLANTS. 14.038, 18.020

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING OIHYOROFOLATE REDUCTASE. 23.053

INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE ANO JACK PINES. **06.004**

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATEO ATRAZINE TOLERANCE IN CORN. 14.069

MOLECULAR GENETIC APPROACHES FOR GERMPLASM
OEVELOPMENT OF FRUIT AND NUT CROPS. 10.001

THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN AND COTTON, 14.068, 21.007*

HERBICIDE-RESISTANCE-(PLANTS)

MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067 HERBICIDES

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING OIHYOROFOLATE REDUCTASE. 23.053

GLYPHOSATE OEGRAOATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE ANO JACK PINES. **06.004**

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069

HERITABILITY

GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065

MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*

HESSIAN-FLY

GENETICS OF BIOTYPES IN THE HESSIAN FLY (MAYETIOLA DESTRUCTOR). 17.014

HETEROCYSTS

STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED DURING NITROGEN FIXATION. 67.084

HETEROKARYONS

GENETICS OF ASPERGILLUS FLAVUS. **67.036 HETEROSIS**

DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056 HETEROZYGOSITY

GENETICS OF ASPERGILLUS FLAVUS. 67.036

HEXAPLOIDS

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*

CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002

GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOID WHEAT AND ITS RELATIVES. 67.097

SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEOICAGO. 20.001

HIGH-LYSINE-CORN

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

HOLLOW-HEART-(POTATOES)

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH ANO VARIETAL IMPROVEMENT. 11.010

HOMOLOGY

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC
MALE STERILE AND FERTILE PLANTS, 62,005

MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067

ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. 67.092

HOMOSERINE

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

HORIZONTAL-RESISTANCE

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE AND FOOD VALUE. 12.021

HORMONAL-RELATIONSHIPS

CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002

HORMONE-TREATMENT

GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003*

HORMONE,

REGULATION OF GRASSHOPPER REPRODUCTION BY JUVENILE HORMONE. **67.023**

HORTICULTURAL-CROPS

OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING DISEASE RESISTANCE INTO POTATOES. 11.018

HOST-PARASITE-RELATIONS

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018, 07.001. 12.048*, 14.079*, 17.023*, 18.029*

HOST-PATHOGEN-RELATIONS

ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. 06.015

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012* HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM:

BACTERIAL BLIGHT OF RICE. 16.002 HOST-RANGE

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012* IMPROVING THE EFFICACY OF BACULOVIRUS

PESTICIDES BY RECOMBINANT ONA TECHNOLOGY. 65.002, 67.031*

HOST-RESISTANCE

PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAD OISEASES OF FRUIT

TREES. 09.006, 10.004*, 66.005* HOST-RESPONSE SOYBEAN BROWN STEM ROT. 23.008 HOST-SPECIFICITY GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018, 12.048*, 14.079* HPLC-(CHROMATOGRAPHY) PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. 67.041 CELL WALLS OF MAIZE PERICARP. 15.010 CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS. 14.032, 23.024*, 30.001*, 31.002* HYBRIDIZATION A PLANT MITOCHONDRIAL MATURASE GENE. 67.099 CELL SPECIFIC GENE EXPRESSION DURING LATICIFER DIFFERENTIATION IN POPPY. 67.096 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010 CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA DNA. 06.011 CHILLING AND GENE EXPRESSION IN FRUIT. 12.006 CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032* CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIDIZE TO UNIQUE-SEQUENCE-ONA CLONES. COSMIO MAPPING OF MITOCHONORIAL ONA OF MALE FERTILE AND MALE STERILE SORGHUM. 15.002 CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009 CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015 DEVELOPMENT OF A ONA HYBRIDIZATION ASSAY FOR DETECTION OF THE RING ROT PATHOGEN. 11.007 DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056 EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIDIZING POPULATIONS. 14.051 EXPLOITATION OF RIBOSOMAL DNA TO CONTROL PLANT PATHOGENIC FUNGI. 67.101 GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013 GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004* GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007* MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016* MOLECULAR MAPPING OF GENES IN CORN. 14.040 MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021 ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017 PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.082 STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043 TOWARDS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017 USE OF SINGLE COPY CLONED ONA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028 VEGETABLE GENETICS. 12.014 HYBRIDIZATION,

BASIC AND APPLIED MOLECULAR GENETICS OF THE

POTATO BACTERIAL RING ROT PATHOGEN. 11.008

HYBRIDOMAS

FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034
PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004
HYRRIDS

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043

GENETICS AND BREEDING OF COOL SEASON CROPS.
12.011

HYBRIO VARIEGATION IN PHASEOLUS VULGARIS. 23.038

INHERITANCE AND PHENDTYPIC SIGNIFICANCE OF CHLOROPLAST DNA POLYMORPHISMS IN LOOGEPOLE AND JACK PINES. **06.004**

MITOCHONDRIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. **67.030**

STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREDII, A NEWLY DESCRIBED SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

HYDROGEN-UPTAKE

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*, 70.001*

HYDROLASES

ORGANIZATION AND CONTROL OF THE HYDROLASE GENES IN BARLEY. 18.018

HYDROPONICS

MODULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021

HYDROXYLASES

PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. **67.041**

HYPERSENSITIVITY

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*

HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113

MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

HYPOVIRULENCE

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. **06.018**, **12.048***, **14.079***

IMBIBITIONAL-FRACTURE

BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006*

IMBIBITIONAL-STRESS

BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006*

IMMUNOCHEMISTRY

GENES FOR PHOTOSYNTHESIS IN CORN. $\mathbf{67.058}$ **IMMUNOLOGY**

BACTERIAL GENES COOING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. **67.098**

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 IN-VITRO

EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*

FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. **67.020**

GENETICS AND CYTOLOGY OF MAIZE. 14.022
GENETICS AND MOLECULAR BIOLOGY OF COTTON
CYTOPLASMIC MALE STERILITY. 21.003

CYTOPLASMIC MALE STERILITY. 21.003
MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC
MALE STERILE AND FERTILE PLANTS. 62.005

PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.016

SYSTEMS FOR THE MOLECULAR ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

IN-VIVO

BACTERIAL GENES CODING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098

INBREDS

CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
EVALUATION AND ENHANCEMENT OF WHEAT AND OAT
ACCESSIONS IN THE NATIONA L SMALL GRAINS
COLLECTIO. 17.008, 18.011*

ISOLATION OF TRANSPOSON INOUCED MAIZE MUTANTS OFFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

INBREEDING

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043

GENETIC MECHANISMS IN CORN. 14.044

ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058

INCOMPATIBILITY

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018*

GENETICS OF ASPERGILLUS FLAVUS. **67.036**ISOLATING ANO CHARACTERIZING A-BETA
MATING-TYPE ALLELES OF SCHIZOPHYLLUM
COMMUNE.. **66.013**, **67.102***

INDUCED-RESISTANCE

GENETICS AND BREEDING OF COOL SEASON CROPS.
12.011

INDUCERS

OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015

INFECTION

EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*

INFECTIVITY

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*

INHERITANCE

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME ANO ONA MARKERS. 12.041

GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006
GENETIC CONTROL OF SEMIGAMY; AND DERIVATION
OF NULLISOMIC COTTON. 21.013

GENETIC STRUCTURE OF CULTIVATEO SOYBEANS AND THE WILD SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045

GENETICS ANO IMPROVEMENT OF CORN USING MOLECULAR AND TRAOITIONAL METHOOS. 14.057 GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON

GENE MAPPING. 23.016
GENETICS, CYTOGENITCS OF COTTON GERMPLASM.
21.008

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

INHERITANCE OF MITOCHONORIAL ONA IN SOMACLONAL VARIANTS. 20.003

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI.

14 058

MAPPING, TRANSFER, RECOMBINATION AND

EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035

SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013

INHIBITION

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

INHIBITORS

BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033
DIFFERENTIAL EXPRESSION OF PROTEINASE
INHIBITOR GENES IN PLANT TISSUES. 11.015
STRUCTURE, EVOLUTION AND FUNCTION OF PLANT
PROTEINASE INHIBITORS. 11.016, 12.044*,
20.014*, 26.006*, 63.006*

INJURIES

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

INOCULUM

GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVED AGRONOMIC PROPERTIES. 23.039

STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREDII, A NEWLY DESCRIBED SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

INSECT-BEHAVIOR

IMPROVING THE EFFICACY OF BACULOVIRUS
 PESTICIOES BY RECOMBINANT ONA TECHNOLOGY.
65.002, 67.031*

INSECT-CONTROL

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. 06.015

GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003*

GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*,

21.005*, 23.040*

METHODS OF CONTROLLING CORN INSECTS. 14.024

INSECT-ECOLOGY

METHODS OF CONTROLLING CORN INSECTS. 14.024

METHOOS OF CONTROLLING CORN INSECTS. 14.024
INSECT-GENETICS

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051

GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*, 21.005*. 23.040*

INSECT-PARASITES

GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*, 21.005*, 23.040*

INSECT-PATHOGENS

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. 06.015

INSECT-PHEROMONES

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051

INSECT-POPULATION

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051

INSECT-VIRUSES

IMPROVING THE EFFICACY OF BACULOVIRUS
 PESTICIDES BY RECOMBINANT ONA TECHNOLOGY.
65.002, 67.031*

INSECTICIDE-RESISTANCE

POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS. 21.004

INSECTICIDES

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018.

12.048*. 14.079*

METHOOS OF CONTROLLING CORN INSECTS. 14.024 INSECTS

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. 06.015

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051

GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS ANO OISEASES. 06.003

IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIOES BY RECOMBINANT ONA TECHNOLOGY. 65.002, 67.031*

METHODS OF CONTROLLING CORN INSECTS. 14.024 STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

INTERGENERIC-HYBRIDIZATION

WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

INTERPLOID-CROSSES.

EXPLORATORY RESEARCH ON THE CYTOGENETICS OF SOLANACEOUS CROPS. 11.001

INTERSPECIFIC

OEVELOPMENT OF A MOLECULAR CYTOGENETIC MAP OF COTTON BY IN SITU HYBRIOIZATION. 21.012

INTERSPECIFIC-HYBRIDIZATION

CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011

GENOME ORGANIZATION IN THE CULTIVATED AND WILO SPECIES OF TOMATO. 12.015

SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

INTERSPECIFIC-HYBRIDS

EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015 SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

INTERSPECIFIC-VARIATION

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047

INTRASPECIFIC-VARIATION

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047 USE OF MOLECULAR MARKERS IN PLANT BREEDING

ANO GENETICS. 67.071

INTRON

A PLANT MITOCHONORIAL MATURASE GENE. 67.099 **INTRONS**

MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011

IODINE

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. 67.044

IRON-BINDING-PROTEINS

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. 67.044

IRRADIATION

ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

IRRIGATION

GERMPLASM ENHANCEMENT AND IMPROVEO BREEDING METHOO S IN BARLEY. 18.001

ISOELECTRIC-FOCUSING

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIOANTS IN CORN. 14,014

EFFICIENCY OF NITROGEN FIXATION. 23.025. 66.003*

ISOENZYME

THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIDASE. 26.005

ISOENZYMES

GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. 06.007

ISOGENIC-LINES

DEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELD AND GENE EXPRESSION. 67.018

ISOLATES

GENETICS OF ASPERGILLUS FLAVUS. 67.036

ISOLATION

CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF OS ELEMENT. 14.053

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.010

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017. 23.010*

ISOLATION OF GENES THAT ENCODE ONA BINOING PROTEINS. **67.066**

ISOLATION OF TRANSPOSON INOUCEO MAIZE MUTANTS OEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067 REGULATION OF TUBER PROTEIN SYNTHESIS IN

POTATO. 11.014, 67.094* SOYBEAN BROWN STEM ROT. 23.008

THE ROLE OF POLYAMINES OURING STRESS-INDUCEO ALTERATIONS IN GENE EXPRESSION. 67.073

ISOSOMES

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001

ISOZYME-LINKAGE

BREEDING, DISEASES AND CULTURE OF DRY PEAS ANO LENTILS. 12.040, 62.006*

ISOZYMES

CELLULAR GENETICS OF CITRUS SPECIES. 09.002 CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*

CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002

OEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELO AND GENE EXPRESSION. 67.018

DISSECTION OF HETEROSIS OF QUANTITATIVE

TRAITS USING MOLECULAR MARKERS. 14.056
ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039, 12.041

EVALUATION OF GENETIC VARIABILITY IN ECHINACEA. 25.005, 28.003*

EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT

GENETICS RESEARCH. 12.032 FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034

GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOID WHEAT AND ITS RELATIVES. 67.097

GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

GENETIC MOSAICS AND THEIR RELATIONSHIP TO

PATTERNS OF SUSCEPTIBILITY TO INSECTS AND OISEASES. 06.003

GENETIC STRUCTURE OF CULTIVATEO SOYBEANS AND THE WILO SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045

GENETIC STRUCTURE OF WEEO-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPODIUM). 14.073

GENOME EVOLUTION IN BRASSICA. 12.012
GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELDIDOGYNE INCOGNITA, 67.053

ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058, 14.063

ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080

MAPPING THE SYMBIOSIS GENES OF PEA. **67.077**MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. **14.060**

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATEO ATRAZINE TOLERANCE IN CORN. 14.069

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006

MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021

PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.016

RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN. 23.017

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. 67.044

SYSTEMS FOR THE MOLECULAR ANALYSIS OF OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007
THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN
AND COTTON. 14.068, 21.007*

USE OF MOLECULAR MARKERS IN PLANT BREEDING AND GENETICS. 67.071

VEGETABLE GENETICS. 12.014

JUVENILE.

REGULATION OF GRASSHOPPER REPRODUCTION BY JUVENILE HORMONE. 67.023

JUVENILES

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. **06.009**

KARYOTYPE

BARLEY GENETICS AND PLANT CYTOGENETICS.
18.008, 20.004*, 27.001*, 67.022*
KARYOTYPES

CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. **06.016**

KERNEL-BREAKAGE

MAIZE GRAIN PROTEIN COMPOSITION AND OISTRIBUTION AS RELATED TO HARONESS, HANOLING AND PROCESSING. 14.016

KERNEL-QUALITY

MAIZE GRAIN PROTEIN COMPOSITION AND OISTRIBUTION AS RELATED TO HARONESS, HANDLING AND PROCESSING. 14.016

KERNEL-SIZE

ISOLATION OF GENES FOR QUANTITATIVE

INHERITANCE IN MAIZE. 14.030

KERNEL-SIZE,

ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.029

KINETICS

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069

KLEBSIELLA-PNEUMONIAE

EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*

LACTATE-DEHYDROGENASE

GENETIC MANIPULATION OF LACTIC OEHYOROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE.
18.019

LACTIC-DEHYDROGENASE

GENETIC MANIPULATION OF LACTIC OEHYOROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019

LARVAE

METHOOS OF CONTROLLING CORN INSECTS. 14.024 LATICIFERAE

CELL SPECIFIC GENE EXPRESSION OURING
LATICIFER OIFFERENTIATION IN POPPY. 67.096

LAURIC-ACID

OEVELOPMENT OF NEW AND IMPROVED CROPS FOR WATER CONSERVATION IN ARIO LANOS. 25.004

LEAF-ANALYSIS

CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014

LEAVES

OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015
GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

HYBRIO VARIEGATION IN PHASEOLUS VULGARIS. 23.038

REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037

SOYBEAN BROWN STEM ROT. 23.008 STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043

LECTINS

GENE EXPRESSION IN PLANT OEVELOPMENT. 23.033
REGULATION OF SOYBEAN SEED PROTEIN GENE
EXPRESSION. 23.001

STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE. 23.035

LEGHEMOGLOBIN

ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. 67.092

LEGUMES

AMINO ACIO METABOLISM ANO THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*

CONTROL OF THE BIOSYNTHESIS OF PROTEINS &
AMINO AC IOS IN LEGUME SEEDS. 23.046
GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF

F000 LEGUMES. 12.043, 62.007*

LENGTH

GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

LENGTH-POLYMORPHISMS

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN SORGHUM. 15.009

LENS

GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

LENTILS

BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006*

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF F000 LEGUMES. 12.043, 62.007*

LENTINULA-EDODES

GERMPLASM ENHANCEMENT AND CULTURE OF EOIBLE MUSHROOMS. 12.038

LEPIDOPTERA

IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIOES BY RECOMBINANT ONA TECHNOLOGY. 65.002, 67.031*

LESIONS

CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. 67.014

GENETIC CONTROL OF LEAF LESION FORMATION IN MAIZE. 14.047

REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003

LETTUCE

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH ANO PRODUCTION EFFICIENCY. 12.010 VARIATION IN LETTUCE COWNY MILOEW. 12.008 LETTUCE,

ORGANIZATION AND STABILITY OF GENES FOR RESISTANCE AND AVIRULENCE. 67.013

CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIDIZE TO UNIQUE-SEQUENCE-ONA CLONES. 17.031

OEVELOPMENT OF A ONA HYBRIDIZATION ASSAY FOR OETECTION OF THE RING ROT PATHOGEN. 11.007

FUNCTION OF PLANT RNA-OEPENOENT RNA

POLYMERASES. 12.036

ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015

ISOLATION OF GENES THAT ENCODE ONA BINDING PROTEINS. **67.066**

ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. 62.001

LIFE-CYCLE

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004

ISOLATION AND CHARACTERIZATION OF GENES REGULATEO BY LIGHT. 67.080

MOLECULAR AND GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION ANO EXPRESSION. 18.036, 63.007*

LIGHT-ABSORPTION

FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOID PROTEIN. **67.020**

LIGHT-INTENSITY

PHOTOREGULATED GENE EXPRESSION IN CHLOROPLASTS. 67.081

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

LIGHTING

PHOTOREGULATED GENE EXPRESSION IN CHLOROPLASTS. 67.081

CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*

MOLECULAR BIOLOGY OF CELLULOSE ANO LIGNIN OEGRADATION BY PHANEROCHAETE

CHRYSOSPORIUM. 06.017 THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIDASE. 26.005

LIGNIN-BIOSYNTHESIS

ISOLATION OF A LIGNIN-BIOSYNTHESIS GENE FROM LOBLOLLY PINE. 06.010

LIGNIN-PEROXIDASES

MOLECULAR BIOLOGY OF CELLULOSE AND LIGNIN OEGRADATION BY PHANEROCHAETE CHRYSOSPORIUM. 06.017

LIMNANTHES

PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, AND OISTRIBUTION. 25.001

ITNES

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

RFLP METHODOLOGY FOR RELATEONESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

LINKAGE-(GENETICS)

ANEUPLOIO ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007

CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015

OEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. **67.024**

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039

FURTHER OEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034

GENETIC CONTROL OF LEAF LESION FORMATION IN MAIZE. 14.047

GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014, 21.015

GENETIC STRUCTURE OF CULTIVATED SOYBEANS AND THE WILD SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015 GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATOOE MELOIDOGYNE INCOGNITA. 67.053 MAPPING THE SYMBIOSIS GENES OF PEA. 67.077

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060 MOLECULAR MARKERS IN MAIZE BREEDING AND

v i

GENETICS. 14.021

PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016

RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN. 23.017

STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE & THEIR USE AS RESEARCH TOOLS. 14.018 VEGETABLE GENETICS. 12.014

LINKAGE-CONSERVATION

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND DNA MARKERS. 12.041 LINKAGE-GROUPS

DEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002 GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013
GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

LINKAGE-MAP,

MOLECULAR MARKERS FOR BRASSICA CAMPESTRIS CHROMOSOMES. 12.026

LINKAGE-MAPS

BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006*

LIPID-BODIES, TRAICYLGLYCEROL-S

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.005

LIPID-GENE-EXPRESSION,

STRUCTURE OF GENES INVOLVED IN DIL SYNTHESIS IN MAIZE. 14.005

LIPID-GENES,

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.005

LIPID-METABOLISM

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.072

LIPID-SYNTHESIS

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.072

LIPIDS

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIDS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

REGULATION OF FATTY ACID SYNTHESIS PROTEINS IN SOYBEAN. 23.037

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.072

LIPOPOLYSACCHARIDES

HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113

LIPOXYGENASE

SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. **67.044**

LOBLOLLY-PINE

CONSTRUCTION OF A SATURATED GENETIC LINKAGE MAP FOR LOBLOLLY PINE. **06.002**

ISOLATION OF A LIGNIN-BIOSYNTHESIS GENE FROM LOBLOLLY PINE. **06.010**

LOCALIZATION

FUNCTION OF PLANT RNA-DEPENDENT RNA
POLYMERASES. 12.036
PHOTOREGULATED GENE EXPRESSION IN

CHLOROPLASTS. 67.082

LOW-TEMPERATURE

EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032

LUCIFERASE

THE MOLECULAR BASIS OF BLACK ROT OF CRUCIFERS. 12.001

LYCOPERSICON-CHILENSE

EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032

LYCOPERSICON-HIRSUTUM

EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032

LYMANTRIA-DISPAR

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. 06.015

LYSINE

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

MAGNAPORTHE

CLONING CULTIVAR SPECIFICITY GENES FROM MAGNAPORTHE GRISEA. 16.010

MAIZE

ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTDNA. 67.043

CELL WALLS OF MAIZE PERICARP. 15.010
CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*

CELLULAR AND MOLECULAR GENETICS FOR IMPROVEMENT OF MAIZE AND FESCUE. 14.071, 20.012*

CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013 CHARACTERIZATION OF THE MAIZE GENOME:

SEQUENCES CODING FOR SPECIFIC GENES. 67.038

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008

CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. **67.014**

DNA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEDLING. 14.070

GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007, 15.001*

GENETIC ANALYSES OF THE MUTATOR SYSTEM OF MAIZE. 14.028

GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065

GENETIC MECHANISMS IN CORN. 14.044
GENETICS AND CYTOLOGY OF MAIZE. 14.022
ISOLATION OF GENES FOR QUANTITATIVE
INHERITANCE IN MAIZE. 14.030

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060 MOLECULAR MAPPING OF GENES IN CORN. 14.040

ORGANELLE DNA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. **20.005**ORGANIZATION OF THE R CHROMOSOME REGION IN

PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. **67.047**

STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036
STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE & THEIR USE AS RESEARCH TOOLS. 14.018
TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*

TRANSPOSABLE ELEMENTS IN MUTATOR ACTIVITY IN MAIZE. 14.026

TUBULIN GENES OF PLANTS. 67.060

MAIZE-GENES,

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.005

MAIZE-GENETICS

MAIZE. 14.077

GENETIC ANALYSES OF THE MUTATOR SYSTEM OF MAIZE. 14.028

MAIZE

ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.029
STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS

IN MAIZE. 14.005
TISSUE CULTURE GENETIC SYSTEMS. 67.059

MALATE

AMINO ACID METABOLISM AND THE RHIZOBIUM-LEGUME SYMBIOSIS. **66.014**, **67.107***

MALE-FERTILITY

COSMIO MAPPING OF MITOCHONDRIAL DNA OF MALE FERTILE AND MALE STERILE SORGHUM. 15.002

MALE-STERILITY

COSMID MAPPING OF MITOCHONDRIAL DNA OF MALE FERTILE AND MALE STERILE SORGHUM. 15.002 VEGETABLE GENETICS. 12.014

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.016

MANGANESE

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

MAP

RFLP METHODOLOGY FOR RELATEDNESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

MAPPING

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN SORGHUM. 15.009

MAPS

PHOTOREGULATED GENE EXPRESSION IN CHLOROPLASTS. 67.082

RFLP METHODOLOGY FOR RELATEDNESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

MARKER-GENES

CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF DS ELEMENT. 14.053
DEVELOPMENT OF A CYTOGENETIC MANIPULATION

SYSTEM FOR COTTON. 21.002

FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060 USE OF MOLECULAR MARKERS IN PLANT BREEDING

AND GENETICS. 67.071

MARKERS

CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE. 27.003

DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034

ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.063

MARKERSRFLP

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

MATHEMATICAL-ANALYSIS

CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS. 14.032, 23.024*, 30.001*, 31.002*

MATING

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018*

MATING-TYPE

ISOLATING AND CHARACTERIZING A-BETA MATING-TYPE ALLELES OF SCHIZOPHYLLUM COMMUNE.. 66.013, 67.102*

MATURASE-GENE

A PLANT MITOCHONDRIAL MATURASE GENE. 67.099 MATURATION

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.072

MATURITY

GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY RECIPROCAL CHROMOSOME SUBSTITUTIONS. 17.024

MECHANISM-OF-ACTION

BIOCHEMISTRY OF PLANT CUTICLE. 67.108 CELLULAR REGULATION OF THE EXPRESSION OF DORMANT GENES IN CEREAL GRAINS. 62.002

CONTROL OF THE BIOSYNTHESIS OF PROTEINS & AMINO AC IDS IN LEGUME SEEDS. 23.046

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043

GENETICS OF FUNGAL PLANT PATHOGENS. 67.051 MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND DEVELOPMENT. 67.015

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. 67.044

MECHANISM-OF-RESISTANCE

MOLECULAR GENETICS OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.005

MEDICAGO

SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

MEDICAGO-SATIVA

SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

MEDIUM-CHAIN-FATTY-ACIDS

DEVELOPMENT OF NEW AND IMPROVED CROPS FOR WATER CONSERVATION IN ARID LANDS. 25.004

MEDIUM-CHAIN-TRIGLYCERIDES

DEVELOPMENT OF NEW AND IMPROVED CROPS FOR WATER CONSERVATION IN ARID LANDS. 25.004

MEIOSIS

BARLEY GENETICS AND PLANT CYTOGENETICS. 18.008, 20.004*, 27.001*, 67.022*
CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN

NORMAL AND MUTANT ZEA MAYS. 67.014

MELOIDOGYNE-INCOGNITA

ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELOIDOGYNE INCOGNITA. 67.053

MEMBRANE-FUNCTION

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046 **MEMBRANE-PROTEINS**

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

MEMBRANE-STRUCTURE

GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003* STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046

MEMBRANES

FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOID PROTEIN. 67.020

REGULATION OF FATTY ACID SYNTHESIS PROTEINS IN SOYBEAN. 23.037

MERISTEMS

GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065

MESOPHYLL-CELLS

CELL SPECIFIC GENE EXPRESSION DURING LATICIFER DIFFERENTIATION IN POPPY. 67.096

METABOLIC-PATHWAYS

AMINO ACID METABOLISM AND THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*

THE ROLE OF POLYAMINES DURING STRESS-INDUCED ALTERATIONS IN GENE EXPRESSION. 67.073

METABOLIC-REGULATION

REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037

THE ROLE OF POLYAMINES OURING STRESS-INDUCED ALTERATIONS IN GENE EXPRESSION. 67.073

METABOLISM

THE PHYTOPATHOGENIC BACTERIA. **67.052 METHIONINE**

CONTROL OF THE BIOSYNTHESIS OF PROTEINS &

AMINO AC IOS IN LEGUME SEEOS. 23.046
MAPPING, TRANSFER, RECOMBINATION ANO
EXPRESSION OF ORGANELLE AND NUCLEAR GENES.
16.004, 17.020*, 23.031*, 67.056*

OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING OISEASE RESISTANCE INTO POTATOES. 11.018

PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. **67.041**

METHYLATION

METHODOLOGY

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING OIHYDROFOLATE REDUCTASE. 23.053

ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEOLING. 14.070

PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028 REGULATION OF PLANT GENE EXPRESSION BY ONA METHYLATION. 67.110

MI-GENE

MOLECULAR APPROACH TO A GENE CONFERRING NEMATOOE RESISTANCE TO TOMATO. **65.001**, **67.012***

MICE

FURTHER OEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034

MICROBIAL-CULTURES

SOYBEAN BROWN STEM ROT. 23.008

MICROBIAL-GENETICS

GENETICS OF ASPERGILLUS FLAVUS. 67.036
STRUCTURE AND REGULATION OF NIF GENES IN
RHIZOBIUM FREOII, A NEWLY DESCRIBED
SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

MICROBIAL-METABOLISM

GLYPHOSATE DEGRAOATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

MICROBIAL-PESTICIDES

IMPROVING THE EFFICACY OF BACULOVIRUS
 PESTICIOES BY RECOMBINANT DNA TECHNOLOGY.
65.002, 67.031*

ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. 65.004, 67.040*

MICROBIOLOGY

CHARACTERIZATION OF NITROGEN FIXATION GENES
AND THEIR PRODUCTS FROM AZOTOBACTER
VINELANOII. 23.057

GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANOII. 23.048, 66.006*

MICROORGANISMS

CELLULAR ANO MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*, 70.001*

MIGRATION

POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS. 21.004

MISSISSIPPI

POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS. 21.004

MITOCHONDRIA

ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. 67.043

BIOCHEMICAL AND DEVELOPMENTAL GENETICS OF HIGHER PLANTS. 14.038, 18.020

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010

COOING AND REGULATION OF MITOCHONDRIAL GENES. $\mathbf{14.052}$

CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIOS AND CYBRIOS OF TOMATO. 12.030

HYBRIOS AND CYBRIOS OF TOMATO, 12.030
COSMIO MAPPING OF MITOCHONORIAL ONA OF MALE

FERTILE AND MALE STERILE SORGHUM. **15.002** OEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. **14.061**

ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEOLING. 14.070

EXPRESSION OF MAIZE MITOCHONORIAL GENOME.

GENETIC MECHANISMS IN CORN. 14.044
INHERITANCE OF MITOCHONDRIAL ONA IN
SOMACLONAL VARIANTS. 20.003

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC
MALE STERILE AND FERTILE PLANTS. 62.005

ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC
MALE STERILITY IN PENNISETUM. 20.005

MITOCHONDRIAL-DNA

A PLANT MITOCHONORIAL MATURASE GENE. **67.099**ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA
MAYS MTONA. **67.043**

COSMIO MAPPING OF MITOCHONDRIAL ONA OF MALE FERTILE AND MALE STERILE SORGHUM. **15.002**

CROP IMPROVEMENT AND GERMPLASM OEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

CYTOPLASMIC FACTORS OF THE POTATO. 11.012 EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051

EXPRESSION OF MAIZE MITOCHONDRIAL GENOME.
14.002

GENETICS OF BIOTYPES IN THE HESSIAN FLY (MAYETIOLA OESTRUCTOR). 17.014

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

MITOCHONORIAL DNA VARIATION IN SUBPOPULATIONS
OF SOMATIC HYBRID CELLS OF GRASSES. 67.030

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005

ORGANELLE DNA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. **20.005**

RECOMBINANT DNA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*

REPEATED ONA SEQUENCES AND CHLOROPLAST DNA INSTABILITY IN CLOVER. 20.008

MODE-OF-ACTION

ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

MODE-OF-INHERITANCE

CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
GENETIC CONTROL OF PLANT MORPHOGENESIS:
STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE.
14.065

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012* HYBRID VARIEGATION IN PHASEOLUS VULGARIS. 23.038

INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST DNA POLYMORPHISMS IN LODGEPOLE AND JACK PINES. **06.004**

STRUCTURE, VARIATION ANO INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036

MODELING-TECHNIQUES

FURTHER OEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034

MODELS

CONSULTATION AND RESEARCH IN MATHEMATICAL AND

- STATISTICAL GENETICS. 14.032, 23.024*,
- 30.001*, 31.002*
 PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. 06.013
- REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061 VARIATION IN LETTUCE ODWNY MILOEW. 12.008
- MOLECULAR
 - CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE. 27.003

MOLECULAR-BIOLOGY

- CELL SPECIFIC GENE EXPRESSION OURING LATICIFER OIFFERENTIATION IN POPPY. 67.096
- CELLULAR AND MOLECULAR GENETICS FOR IMPROVEMENT OF MAIZE AND FESCUE. 14.071,
- CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002
- CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF OS ELEMENT. 14.053
- CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006
- DEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING DISEASE RESISTANCE INTD PDTATOES. 11.018
- EXPRESSION OF MAIZE MITOCHDNDRIAL GENDME. 14.002
- FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATD. 12.034
- GENE EXPRESSIDN IN PLANTS: A STUDY DF POLYADENYLATION IN PLANTS. 20.007
- GENE REGULATION, EXPRESSIDN, AND MDLECULAR MAPPING IN MAIZE AND SDRGHUM. 14.007, 15.001*
- GENES FOR PHOTDSYNTHESIS IN CDRN. 67.058 GENETICS. PHYSIDLDGY AND MDLECULAR BIDLDGY DF HDST-PATHDGEN INTERACTION. 10.008, 66.012*
- GENDME ORGANIZATION IN THE CULTIVATED AND WILD SPECIES DF TOMATD. 12.015
- GLIADIN GENES DF COMMON WHEAT AND ITS ANCESTORS. 17.011
- ISDLATION AND CHARACTERIZATION OF GENES REGULATED BY LIGHT. 67.080
- MANIPULATION OF STDRAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012
- MECHANISMS DIRECTING HDRMDNAL AND DEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026
- MDLECULAR AND GENETIC STUDIES DF NITRATE REDUCTASE IN BARLEY. 18.034
- MDLECULAR CONTRDL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003 MOLECULAR GENETIC ANALYSIS DF THE BRASSICA S
- LOCUS. 67.067
- MOLECULAR SYSTEMATICS OF SDYBEAN (GLYCINE: LEGUMINOSAE). 23.047
- ORGANELLE DNA ORGANIZATION AND CYTDPLASMIC MALE STERILITY IN PENNISETUM. 20.005
- ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENDME. 62.001
- ORGANIZATION AND EXPRESSIDN OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. **67.092**
- PATHOGENSIS, MDLECULAR BIOLOGY AND BIOLDGICAL CONTROL OF XANTHOMDNAD DISEASES OF FRUIT
- TREES. 09.006, 10.004*, 66.005*
 PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH DNA METHYLATIDN. 23.028
- PLASMIDS IN PLANT PATHDGENIC BACTERIA. 18.012, 26.001* STRUCTURE AND EXPRESSION OF SOYBEAN LEAF
- STORAGE PROTEIN GENES. 23.043

- STRUCTURE, EVDLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*
- STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036
- THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001
- THE PHYTOPATHDGENIC BACTERIA. 67.052
- THE REPLICATION OF CHLOROPLAST DNA IN CHLAMYDDMONAS REINHAROII. 67.057
- WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

MOLECULAR-GENETICS

13.002

- ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTDNA. 67.043
- CELLULAR AND MOLECULAR GENETICS FOR CRDP IMPROVEMENT. 14.009, 15.003*, 18.010, 18.013, 23.006*, 24.001*, 26.002*, 67.027, 67.028, 67.035
- CHARACTERIZATION OF PLANT GENES ENCODING PHOTOSYNTHETIC MEMBRANE PROTEINS. 67.074
- CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013
- CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES CDDING FDR SPECIFIC GENES. 67.038
- CLASSICAL GENE MAPPING IN SDYBEAN USING MOLECULAR MARKERS. 23.014
- CLDNING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSDSPDRIUM. 06.012. 66.011*
- CLDNING, EXPRESSIDN AND MUTAGENESIS DF SOYBEAN SEEDLING OIHYDROFDLATE REOUCTASE. 23 053
- DEVELOPMENT OF MOLECULAR BIDLDGY MEANS FOR INCDR- PORATING DISEASE RESISTANCE INTD POTATDES. 11.018
- DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS.
- DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING CDMPLEX GENES. 23.004
- DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MDLECULAR MARKERS. 14.056
- DNA METHYLATION AND CONTROL DF GENE EXPRESSION IN SHOOT AND RODT OF MAIZE SEEDLING. 14.070 EXPRESSIDN DF MAIZE MITOCHDNDRIAL GENOME.
- 14.002
- EXPRESSION OF SDYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007
- FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TDMATD. 12.034
- GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SDRGHUM. 14.007, 15.001*
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY, 06.018, 12.010, 12.048*, 14.079*, 67.104
- GENETIC STRUCTURE AND ADAPTATION DF NATURAL POPULATIONS DF SPRUCE. **06.007**
- GENETICS AND CYTDLDGY DF MAIZE. 14.022 GENETICS AND MDLECULAR BIDLOGY DF CDTTON CYTDPLASMIC MALE STERILITY. 21.003
- GENETICS DF NITRDGEN FIXATION IN AZOTOBACTER VINELANDII. 23.049, 66.007*
- GENETICS, TISSUE CULTURE, AND MDLECULAR BIOLDGY OF THE SDYBEAN. 23.018
- GENOME DRGANIZATION IN THE CULTIVATED AND WILD SPECIES OF TDMATD. 12.015 Inheritance and Phendtypic Significance of
- CHLDROPLAST DNA POLYMORPHISMS IN LOOGEPDLE AND JACK PINES. 06.004

```
ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059
```

ISOLATION AND CHARACTERIZATION OF GENES REGULATED BY LIGHT. 67.080

ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005
MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036
MODULATION AND NITROGEN FIXATION OF PRC R.

JAPONICUM. 23.021

MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN
ANTHERS OF BRASSICA. 67.069

MOLECULAR AND GENETIC STUDIES OF NITRATE

REDUCTASE IN BARLEY. 18.034
MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATEO

ATRAZINE TOLERANCE IN CORN. 14.069
MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE
IN PSEUDOMONAS SYRINGAE PV. GLYCINEA.

23.002, 66.001*

MOLECULAR BIOLOGY OF THE PECTATE LYASE

ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006
MOLECULAR CHARACTERIZATION OF THE SUCROSE

SYNTHETASE-2 GENE OF MAIZE. 14.011
MOLECULAR CLONING OF THE GLUTATHIONE

S-TRANSFERASE GENES FROM CORN. 14.067 MOLECULAR CLONING OF THE RUBBER TRANSFERASE

GENE FROM GUAYULE. 28.004

MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003

MOLECULAR GENETIC APPROACHES FOR GERMPLASM
OEVELOPMENT OF FRUIT AND NUT CROPS. 10.001

MOLECULAR GENETIC CHANGES ASSOCIATEO WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*

MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017

MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA DUCH.. 10.005

MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022

MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*

MOLECULAR MANIPULATION OF GENES. 28.002 MOLECULAR MAPPING OF GENES IN CORN. 14.040

MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM CIVERSITY AND SYSTEMATICS. 12.020

MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021

ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. 20.005

ORGANIZATION AND EXPRESSION OF A-AMYLASE GENES IN THE BARLEY GENOME. 62.001

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.016

RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*

REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNOS IN HIGHER PLANTS. 67.045

REPEATEO ONA SEQUENCES ANO CHLOROPLAST ONA

INSTABILITY IN CLOVER. 20.008

SYSTEMS FOR THE MOLECULAR ANALYSIS OF OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007
THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN
AND COTTON. 14.068, 21.007*

THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001

THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045

THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION. 18.036, 63.007*

THE REPLICATION OF CHLOROPLAST ONA IN CHLAMYOOMONAS REINHAROII. 67.057

TISSUE-SPECIFIC GENE REGULATION IN MAIZE. 14.050

USE OF MOLECULAR MARKERS IN PLANT BREEDING AND GENETICS. **67.071**

WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

MOLECULAR-HYBRIDIZATION

TRANSPOSABLE ELEMENTS IN MUTATOR ACTIVITY IN MAIZE. 14.026

MOLECULAR-MARKER,

ORGANIZATION ANO STABILITY OF GENES FOR RESISTANCE ANO AVIRULENCE. **67.013**

MOLECULAR-MARKERS,

MOLECULAR MARKERS FOR BRASSICA CAMPESTRIS CHROMOSOMES. 12.026

MOLECULAR-STRUCTURE

GENETICS OF NITRATE REDUCTION IN BARLEY. 67.105

MOLECULAR-STUDY

A STUDY OF GENETIC OIVERSITY IN PEANUTS USING CHLOROPLAST AND RRNA MOLECULAR MARKERS. 24.002

MOLECULAR-WEIGHT

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

MONO-OXYGENASES

PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. **67.041**

MONOCLONAL-ANTIBODIES

BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033
BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL
OF VIRUSES AND MOLLICUTES CAUSING DISEASES
OF CORN. 14.066, 66.010*

FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

USE OF MOLECULAR MARKERS IN PLANT BREEDING AND GENETICS. **67.071**

MONOSOMES

OEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001

MONOSOMIC

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

MONOSOMICS

CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010 GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF DURUM AND COMMON WHEAT. 17.025

MORCHELLA-SPP

GERMPLASM ENHANCEMENT AND CULTURE OF EDIBLE MUSHROOMS. 12.038

MOREL

GERMPLASM ENHANCEMENT AND CULTURE OF EDIBLE

MUSHROOMS. 12.038

MORPHOGENESIS

ANEUPLOIO ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007

GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE.

MORPHOLOGICAL-TRAITS

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.041

MORPHOLOGY

THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIDASE. 26.005

MOSAIC-VIRUS

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061

GENETIC/MOLECULAR CHARACTERIZATION OF THE MUTATOR-RELATED CY TRANSPOSABLE ELEMENT SYSTEM OF MAIZE. 14.031

MULTIPLE-TRAIT-SELECTION

CRUCIFER DISEASES. 12.047

MUSHROOMS

GERMPLASM ENHANCEMENT AND CULTURE OF EDIBLE MUSHROOMS. 12.038

MUTABLE

MOLECULAR ANALYSIS OF A SOYBEAN TRANSPOSABLE ELEMENT. 23.023

MUTAGENESIS

CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012

CHARACTERIZATION OF NITROGEN FIXATION GENES AND THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING DIHYDROFOLATE REDUCTASE.

EFFICIENCY OF NITROGEN FIXATION. 23.025. 66.003*

FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. 67.020

GENE EXPRESSION IN PLANTS: A STUDY OF POLYADENYLATION IN PLANTS. 20.007

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 17.023*, 18.029*

GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*

GENETICS OF FUNGAL PLANT PATHOGENS. 67.051 HOST-PATHOGEN RECOGNITION AND DISEASE

RESISTANCE IN PLANTS. 67.113

ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. **23.012**

MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006 MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*

PLASMIOS IN PLANT PATHOGENIC BACTERIA. 18.012, 26.001*

SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND **GENETICS. 23.013**

THE PHYCOCYANIN GENES OF AGMENELLUM QUADRUPLICATUM. 67.088

TOWAROS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

MUTAGENS

GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033 GENETICS, CYTOGENITCS OF COTTON GERMPLASM.

MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 TUBULIN GENES OF PLANTS. 67.060

MUTANTS

AMINO ACIO METABOLISM ANO THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*

ANEUPLOID ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

BIOLOGICAL CONTROL OF PLANT PATHOGENIC

BACTERIA. 10.002, 12.018*
CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF OS ELEMENT. 14.053

CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. 67.014

CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. **16.006**

CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002

GENETIC CONTROL OF LEAF LESION FORMATION IN MAIZE. 14.047

GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065

GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.015

GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015 GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018* GENETICS OF ASPERGILLUS FLAVUS. 67.036

GENETICS OF FUNGAL PLANT PATHOGENS. 67.051

GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANOII. 23.048, 23.049, 66.006*, 66.007*

GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008

HOST-PATHOGEN RECOGNITION AND DISEASE

RESISTANCE IN PLANTS. **67.113** ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIDE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027

ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS DEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

MOLECULAR AND GENETIC STUDIES OF NITRATE REOUCTASE IN BARLEY. 18.034

MOLECULAR GENETICS OF NITROGEN METABOLISM IN

RHIZOBIA. 23.022 MUTANT GENES THAT AFFECT ENDOSPERM DEVELOPMENT IN MAIZE. 14.078

ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. 20.005

- PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016
- THE NATURE OF RESISTANCE TO PLANT VIRUSES.
 12.045
- THE ROLE OF POLYAMINES OURING STRESS-INOUCEO ALTERATIONS IN GENE EXPRESSION. 67.073
 TISSUE CULTURE GENETIC SYSTEMS. 14.041,
 18.022*
- TUBULIN GENES OF PLANTS. 67.060 VEGETABLE GENETICS. 12.014

MUTATION

- ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003
- CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF OS ELEMENT. 14.053
- CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
 GENETIC ANALYSES OF THE MUTATOR SYSTEM OF
 MAIZE. 14.028
- GENETIC CONTROL OF LEAF LESION FORMATION IN MAIZE. 14.047
- GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*
- GERMPLASM ENHANCEMENT AND IMPROVEO BREEDING METHOD S IN BARLEY. 18.001
- MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069
- ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077
- TRANSPOSABLE ELEMENTS IN MUTATOR ACTIVITY IN MAIZE. 14.026

MUTATOR

GENETIC ANALYSES OF THE MUTATOR SYSTEM OF MAIZE. 14.028

MUTATOR.

GENETIC/MOLECULAR CHARACTERIZATION OF THE MUTATOR-RELATED CY TRANSPOSABLE ELEMENT SYSTEM OF MAIZE. 14.031

MYCOPARASITES

ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

MYCOPLASMA

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

MYCOPLASMA-LIKE-ORGANISMS

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING OISEASES OF CORN. 14.035, 15.005*

MYCORRHIZAE

- EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*
- RESEARCH ON THE INTRASPECIFIC VARIATION IN CENOCOCCUM GEOPHILUM FR.. 67.076

MYCORRHIZAL

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

MYCOSPHAERELLA

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

MYCOTOXINS

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018*

NADI-GENE

A PLANT MITOCHONORIAL MATURASE GENE. **67.099** NATURAL-POPULATIONS

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

NATURE-OF-RESISTANCE

- BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*
- OEVELOPMENTAL GENETICS USING THE ALCHOL OEHYOROGENASE GENE-SYSTEM IN MAIZE. 14.004
- MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067

NEMATODE-RESISTANCE

- CHARACTERIZATION OF THE MI LOCUS CONFERRING RESISTANCE TO MELOIOOGYNE INCOGNITA IN TOMATO. 12.022
- ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATOOE MELOIOOGYNE INCOGNITA. 67.053

NEMATODES

- MOLECULAR APPROACH TO A GENE CONFERRING NEMATOOE RESISTANCE TO TOMATO. **65.001**, **67.012***
- RFLP ANO MOLECULAR ANALYSIS OF ROOT KNOT NEMATOOES, NEMATOOE INFECTED PLANTS AND PEACHES. 10.009, 23.054*

NEUROTOXINS

IMPROVING THE EFFICACY OF BACULOVIRUS
 PESTICIOES BY RECOMBINANT ONA TECHNOLOGY.
65.002, 67.031*

NEW-CHEMICALS

METHOOS OF CONTROLLING CORN INSECTS. 14.024 NEW-CROPS

- GENETIC STRUCTURE OF WEEO-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPODIUM).
 14.073
- MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004
- PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, DOCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001

NICOTIANA

- FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. 67.020
- GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*

NITRATE-REDUCTASE

- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 67.104
- GENETICS OF NITRATE REDUCTION IN BARLEY. 67.105
- MOLECULAR ANO GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034

NITROGEN

- EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*
- GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018*
- MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022
- MUTANT GENES THAT AFFECT ENOOSPERM OEVELOPMENT IN MAIZE. 14.078
- PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075

NITROGEN-ASSIMILATION

- GENETICS OF NITRATE REDUCTION IN BARLEY. 67.105
- MUTANT GENES THAT AFFECT ENOOSPERM OEVELOPMENT IN MAIZE. 14.078

NITROGEN-FIXATION

- A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103
- AMINO ACIO METABOLISM ANO THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*,
- CHARACTERIZATION OF NITROGEN FIXATION GENES

ANO THEIR PRODUCTS FROM AZOTOBACTER VINELANDII. 23.057

EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*

GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVED AGRONOMIC PROPERTIES. 23.039

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018, 12.048*. 14.079*

GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*

GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANDII. 23.048, 23.049, 66.006*, 66.007*

MODULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021

MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022

ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. **67.092**

PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075

STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREOII, A NEWLY DESCRIBED SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED OURING NITROGEN FIXATION. 67.084

NITROGEN-FIXING-BACTERIA

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*, 70.001*

CHARACTERIZATION OF NITROGEN FIXATION GENES AND THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057

GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVEO AGRONOMIC PROPERTIES. 23.039

GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANDII. 23.048, 23.049, 66.006*,

MOOULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021

NITROGEN-FIXING-MICROORGANISMS

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103

NITROGEN-METABOLISM

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010, 67.104 MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022

NITROGEN-UTILIZATION

GENETICS OF ASPERGILLUS FLAVUS. 67.036
NITROGENASE

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIDIUM PASTEURIANUM AND OTHER MICROORGANISMS. **67.103**

CHARACTERIZATION OF NITROGEN FIXATION GENES AND THEIR PRODUCTS FROM AZOTOBACTER VINELANDII. 23.057

EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*

NODULATION

EFFICIENCY OF NITROGEN FIXATION. 23.025, 66.003*

MAPPING THE SYMBIOSIS GENES OF PEA. 67.077
MODULATION AND NITROGEN FIXATION OF PRC R.
JAPONICUM. 23.021

NODULES

MAPPING THE SYMBIOSIS GENES OF PEA. 67.077

NODULES-(PLANTS)

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*, 70.001*

NOSEMA

METHOOS OF CONTROLLING CORN INSECTS. 14.024 NUCLEAR-MAGNETIC-RESONANCE

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046
NUCLEAR-POLYHEDROSIS-VIRUS

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. **06.015**

ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. **65.004**, **67.040***

NUCLEAR-RNA

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

NUCLEASES

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008

GENE EXPRESSION IN PLANTS: A STUDY OF POLYADENYLATION IN PLANTS. 20.007

NUCLEI

GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005

PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081

NUCLEIC-ACIDS

PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 67.095

STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036

THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001

NUCLEOTIDES

EXPLOITATION OF RIBOSOMAL ONA TO CONTROL PLANT PATHOGENIC FUNGI. 67.101

STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. **67.044**

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 NULLISOMICS

GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014

GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013

NUTRIENT-DEFICIENCY

ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS OFFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055

NUTRIENT-EXCHANGE

AMINO ACIO METABOLISM AND THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*

NUTRIENTS

AMINO ACIO METABOLISM ANO THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*

NUTS

MOLECULAR GENETIC APPROACHES FOR GERMPLASM DEVELOPMENT OF FRUIT AND NUT CROPS. 10.001

OATS

18.003, 67.006*

ANALYSIS OF SEED GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT

STORAGE PROTEINS. 18.015

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035

THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION. 18.036, 63.007*

TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*

OIL

GENETIC ENGINEERING OF OILSEED SPECIES TO IMPROVE OIL QUALITY. 23.020, 25.003*

OIL-CONTENT

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, ANO NATURAL ANTIOXIOANTS IN CORN. 14.014

OIL-CROPS

MOOULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021

OIL-QUALITY

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.072

OILSEEDS

EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*

STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS IN MAIZE. 14.072

OLIGONUCLEOTIDES

ANALYSIS OF SEEO GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016

GENETIC MANIPULATION OF LACTIC OEHYOROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE.

18.019

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012

MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004

ONCOGENES

REGULATION OF PLANT GENE EXPRESSION BY ONA METHYLATION. **67.110**

ORGANELLE-TRANSFER

TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

ORGANELLES

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043

GENETIC MECHANISMS IN CORN. 14.044

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. 20.005

SYSTEMS FOR THE MOLECULAR ANALYSIS OF OIFFERENTIAL GENE EXPRESSION IN MAIZE ANO OTHER EUKARYOTES. 14.062

ORGANIZATION

ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. 67.092

ORGANOGENESIS

CELLULAR ANO MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*

OSTRINIA-NUBILALIS

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051
METHOOS OF CONTROLLING CORN INSECTS. 14.024

OXYGEN

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

PAPAVER-SOMNIFERUM

CELL SPECIFIC GENE EXPRESSION OURING
LATICIFER OIFFERENTIATION IN POPPY. 67.096

PAPAYAS

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

PARAMUTATION

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077

PARASEXUALITY

GENETICS OF ASPERGILLUS FLAVUS. 67.036

PARASITISM

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

PATATIN

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*, 67.095

PATHOGEN

CHARACTERIZATION OF THE MI LOCUS CONFERRING RESISTANCE TO MELOIOOGYNE INCOGNITA IN TOMATO. 12.022

CLONING CULTIVAR SPECIFICITY GENES FROM MAGNAPORTHE GRISEA. 16.010

PATHOGEN-BIOLOGY

HOST-PATHOGEN RECOGNITION AND OISEASE RESISTANCE IN PLANTS. 67.113

PATHOGEN-CHARACTERIZATION

CRUCIFER OISEASES. 12.047

PATHOGEN-IDENTIFICATION

PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO DISEASES OF FRUIT TREES. **09.006**, **10.004***, **66.005***

PATHOGEN-INTERACTIONS

BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*

PATHOGEN-VARIABILITY

CRUCIFER OISEASES. 12.047

PATHOGEN,

BASIC ANO APPLIEO MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN. 11.008

PATHOGENESIS

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010

CELLULAR ANO MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.035

DEFINING ANO MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010

GENETICS OF FUNGAL PLANT PATHOGENS. 67.051

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*

MOLECULAR ANO GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034

PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011
SOYBEAN BROWN STEM ROT. 23.008

PATHOGENICITY

CELLULAR ANO MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.013, 26.002*

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE AND FOOD VALUE. 12.021

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034.

15.004*, 17.018*
GENETICS OF FUNGAL PLANT PATHOGENS. 67.051 MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034

MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046. 67.109*

PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

SOYBEAN BROWN STEM ROT. 23.008

THE PHYTOPATHOGENIC BACTERIA. 67.052

ROLE OF LOW MOLECULAR WEIGHT HEAT SHOCK PROTEINS IN PLANT OEVELOPMENT. 67.002

PEACHES

RFLP AND MOLECULAR ANALYSIS OF ROOT KNOT NEMATODES, NEMATODE INFECTED PLANTS AND PEACHES. 10.009, 23.054*

PEANUTS

A STUDY OF GENETIC DIVERSITY IN PEANUTS USING CHLOROPLAST AND RRNA MOLECULAR MARKERS. 24.002

PEARLMILLET

MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017

CHARACTERIZATION OF PLANT GENES ENCOOING PHOTOSYNTHETIC MEMBRANE PROTEINS. 67.074 GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOO LEGUMES. 12.043, 62.007*

ISOLATION AND CHARACTERIZATION OF GENES REGULATED BY LIGHT. 67.080

MAPPING THE SYMBIOSIS GENES OF PEA. 67.077 PHOTOREGULATEO GENE EXPRESSION IN

CHLOROPLASTS. 67.081, 67.082

PECTATE-LYASE

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006 PECTIN

BIOCHEMISTRY OF PLANT CUTICLE. 67.108

PECTOLYTIC-ENZYMES

THE PHYTOPATHOGENIC BACTERIA. 67.052

PENNISETUM-AMERICANUM

ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC MALE STERILITY IN PENNISETUM. 20.005

PENNISETUM-PURPUREUM

MOLECULAR MANIPULATION OF GENES. 28.002 **PEPPERS**

USE OF MOLECULAR MARKERS IN PLANT BREEDING ANO GENETICS. 67.071

PEPTIDES

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACID SEQUENCING AND ELECTROPHORETIC STUDIES.

17.004, 18.005*
FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. 67.020

PERENNIAL-LEGUMES

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

PEROXIDASE

THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIDASE. 26.005

PEROXIDASES

CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*

PERSISTENCE

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

PEST-CONTROL

GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003* PEST-MANAGEMENT

IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIOES BY RECOMBINANT ONA TECHNOLOGY. 65.002, 67.031*

PESTICIDE-DEGRADATION

GLYPHOSATE DEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

PESTICIDE-METABOLISM

GLYPHOSATE DEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

PESTICIDE-TOLERANCES

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069

PESTICIDES

GLYPHOSATE DEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIDES BY RECOMBINANT DNA TECHNOLOGY. 65.002, 67.031*

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

PETUNIA

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005 USE OF MOLECULAR MARKERS IN PLANT BREEDING ANO GENETICS. 67.071

PHANEROCHAETE

MOLECULAR BIOLOGY OF CELLULOSE AND LIGNIN **OEGRADATION BY PHANEROCHAETE** CHRYSOSPORIUM. 06.017

PHANEROCHAETE-CHRYSOSPORIUM

CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*

PHENOTYPES

CYTOPLASMIC FACTORS OF THE POTATO. 11.012 GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033 INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE AND JACK PINES. 06.004

ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027

MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*

MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*

PHENYLALANINE

BIOCHEMISTRY OF PLANT CUTICLE. 67.108

PHIALOPHORA-GREGATUM

SOYBEAN BROWN STEM ROT. 23.008

PHOSPHOGLUCOSE-ISOMERASE

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 **PHOSPHORYLATION**

THE THYLAKOIO ENERGY TRANSOUCING ATPASE COMPLEX. 67.111

PHOTOCHEMISTRY

PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081

PHOTOLYSIS

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

PHOTORECEPTORS

THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION. 18.036, 63.007*

PHOTOREGULATION

PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081

PHOTOSTIMULATION

PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.082

PHOTOSYNTHESIS

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

CHARACTERIZATION OF PLANT GENES ENCOOING
PHOTOSYNTHETIC MEMBRANE PROTEINS. 67.074
CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS
AND CYTOGENETICS. 14.043

OEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004

GENES FOR PHOTOSYNTHESIS IN CORN. **67.058**GENETICS AND IMPROVEMENT OF NORTHEASTERN
TREES. **06.009**

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. **67.046**STRUCTURE, EXPRESSION AND EVOLUTION OF
CYANOBACTERIAL GENES REGULATED OURING
NITROGEN FIXATION. **67.084**

THE THYLAKOIO ENERGY TRANSOUCING ATPASE COMPLEX. 67.111

PHOTOSYSTEM-I

91

40 7

1

GENES FOR PHOTOSYNTHESIS IN CORN. 67.058

PHOTOSYSTEM-II

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

PHYCOCYANINS

THE PHYCOCYANIN GENES OF AGMENELLUM QUADRUPLICATUM. 67.088

PHYLOGENETIC

SYSTEMATICS OF POTATOES AND THEIR WILD RELATIVES (SOLANUM SECT. PETOTA). 11.017 PHYLOGENY

CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. **06.016**

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047

PHYMATOTRICHUM-OMNIVORUM

ECOLOGY ANO BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

PHYSICAL-MAPPING

TAGGING PLANT GENES WITH TIGHTLY-LINKED RFLP MARKERS. 12.035

PHYSIOLOGICAL-RESPONSE

ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

PHYSIOLOGICAL-STRESS

MITOCHONORIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. **67.030**

PHYSIOLOGY

THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIDASE. 26.005

PHYTOALEXINS

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*

PHYTOCHROME

ISOLATION AND CHARACTERIZATION OF GENES REGULATED BY LIGHT. **67.080**

THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION. 18.036, 63.007*

PHYTOHORMONES

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*

PICEA-ENGELMANNI

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

PICEA-GLAUCA

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

PICEA-PUNGENS

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

PIGMENTATION

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077

PIGMENTS

THE PHYCOCYANIN GENES OF AGMENELLUM QUADRUPLICATUM. 67.088

PINUS-BANKSIANA

INHERITANCE AND PHENDTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE AND JACK PINES. **06.004**

PINUS-CONTORTA-LATIFOLIA

INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE ANO JACK PINES. 06.004

PINUS-PONDEROSA

GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014

PINUS-RADIATA

CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. 06.011

PINUS-STROBUS

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009

PISUM

GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

PLANT

CELL WALLS OF MAIZE PERICARP. 15.010
CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE.
27.003

OEVELOPMENT OF A MOLECULAR CYTOGENETIC MAP OF COTTON BY IN SITU HYBRIOIZATION. 21.012
REGULATION OF PLANT GENE EXPRESSION BY CELL

TURGOR OR ABSCISIC ACIO. 67.093

PLANT-ACCESSIONS

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND ONA MARKERS. 12.039

PLANT-ADAPTATION

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

PLANT-ANATOMY

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 67.095

PLANT-BIOCHEMISTRY

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

BIOCHEMISTRY OF PLANT CUTICLE. 67.108

CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF DS ELEMENT. 14.053

CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043

OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING DISEASE RESISTANCE INTO POTATOES. 11.018

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO

- SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*
- FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOID PROTEIN. 67.020
- GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005
- GENETIC MECHANISMS IN CORN. 14.044
- GENETIC STRUCTURE AND ACAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**
- GENETICS AND CYTOLOGY OF MAIZE. 14.022
- GENOME ORGANIZATION IN THE CULTIVATED AND WILD SPECIES OF TOMATO. 12.015
- ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*
- MOLECULAR CONTROL OF GENE ACTIVITY DURING REPRODUC TIVE OEVELOPMENT IN THE WHEAT PLANT. 17.003
- ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013
- PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. **67.047**
- PHOTOREGULATED GENE EXPRESSION IN CHLOROPLASTS. 67.081
- PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS.

 18 035
- PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001
- REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. **67.095**
- RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023
- REPEATED ONA SEQUENCES ANO CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008
 SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY ANO
- SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013
- STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. **67.044**
- STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046 STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*,
- 20.014*, 26.006*, 63.006*
 STRUCTURE, EXPRESSION AND EVOLUTION OF
 CYANOBACTERIAL GENES REGULATED DURING
 NITROGEN FIXATION. 67.084
- THE THYLAKOIO ENERGY TRANSOUCING ATPASE COMPLEX. 67.111

PLANT-BIOLOGY

- ANALYSIS OF SEEO GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016
 GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033
- MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS DF BRASSICA. **67.069**
- MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067

PLANT-BREEDING

- 18.003, 67.006*
- BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006*
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. **67.035**
- CELLULAR GENETICS OF CITRUS SPECIES. **09.002** CHROMOSOMAL MAPPING OF GENES CONTROLLING
- TISSUE CULTURE RESPONSE IN WHEAT. 17.010
- CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
 CLASSICAL GENE MAPPING IN SOYBEAN USING
 MOLECULAR MARKERS. 23.014
- CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIOS AND CYBRIOS OF TOMATO. 12.030 CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS

- AND CYTOGENETICS. 14.043
- CRUCIFER DISEASES. 12.047
 CYTOPLASMIC FACTORS OF THE POTATO. 11.012
- DEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELD AND GENE EXPRESSION. **67.018**
- DISSECTION OF HETEROSIS OF QUANTITATIVE
 TRAITS USING MOLECULAR MARKERS. 14.056
- FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034
- GENE MAPPING IN SOYBEAN WITH MDLECULAR MARKERS. 23.056
- GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS DF DURUM AND COMMON WHEAT. 17.025
- GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014. 21.015
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010 GENETICS AND BREEDING OF COOL SEASON CROPS.
- GENETICS AND BREEDING OF COOL SEASON CRDPS.
 12.011
- GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS. 14.057
- GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008
- GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018
- GENOME ORGANIZATION IN THE CULTIVATEO AND WILO SPECIES OF TOMATO. 12.015
- GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001
- INHERITANCE OF MITDCHDNORIAL ONA IN SOMACLONAL VARIANTS. 20.003
- ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE FUNCTION AND REGULATION. 14.054
- ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*
- ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058
- ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGDSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080
- MAIZE GRAIN PROTEIN COMPOSITION AND DISTRIBUTION AS RELATED TO HARDNESS, HANDLING AND PROCESSING. 14.016
- MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF DRGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*
- MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060
- MODULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021
- MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003
- MOLECULAR GENETIC APPROACHES FOR GERMPLASM DEVELOPMENT OF FRUIT AND NUT CROPS. 10.001
- MDLECULAR GENETIC STUDIES IN THE CULTIVATEO STRAWBERRY, FRAGARIA X ANANASSA DUCH.. 10.005
- MOLECULAR MAPPING OF GENES IN CORN. 14.040
 MOLECULAR MARKERS IN MAIZE BREEDING AND
 GENETICS. 14.021
- MOLECULAR SWITCHES IN PLASTIO DIFFERENTIATION. 67.089
- MUTANT GENES THAT AFFECT ENOOSPERM OEVELOPMENT IN MAIZE. 14.078
- PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001

- PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001, 21.016
- RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN. 23.017
- TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*
- TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*
- TISSUE-SPECIFIC GENE REGULATION IN MAIZE. 14.050
- VEGETABLE GENETICS. 12.014

PLANT-CHARACTERISTICS

CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014

PLANT-COLLECTION

CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIOS AND CYBRIOS OF TOMATO. 12.030

PLANT-CYTOGENETICS

- CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043
- DEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002
- DEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. 14.061
- GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF DURUM AND COMMON WHEAT. 17.025
- GENETICS AND CYTOLOGY OF MAIZE. 14.022
- GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003
- GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008
- GENOME EVOLUTION IN BRASSICA. 12.012
- GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001
- PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. 67.041
- STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE & THEIR USE AS RESEARCH TOOLS, 14.018 TISSUE CULTURE GENETIC SYSTEMS. 14.041,
 - 18.022*

PLANT-CYTOLOGY 18.003, 67.006*

- GENETICS AND CYTOLOGY OF MAIZE. 14.022 MEASURING THE GENETIC OIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060
- PLANT-DEVELOPMENT
 - CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010, 67.027
 - CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046 DEVELOPMENTAL GENETICS USING THE ALCHOL
 - DEHYDROGENASE GENE-SYSTEM IN MAIZE. 14.004 GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005
 - GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065
 - GENETIC ENGINEERING TO IMPROVE PLANT HEALTH ANO PRODUCTION EFFICIENCY. 67.104
 - MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND DEVELOPMENT. 67.015
 - MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA DUCH... 10.005
 - ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055
 - THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION ANO EXPRESSION. 18.036, 63.007*
- THE ROLE OF POLYAMINES OURING STRESS-INDUCED ALTERATIONS IN GENE EXPRESSION. 67.073
- PLANT-DEVELOPMENTAL-BIOLOGY
 - GENETIC ENGINEERING OF OILSEED SPECIES TO IMPROVE OIL QUALITY. 23.020, 25.003*

PLANT-DISEASE-CONTROL

- BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*
- BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*
- BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*
 CRUCIFER OISEASES. 12.047
 GENETIC IMPROVEMENT OF BEANS (PHASEOLUS
- VULGARIS L.) FOR YIELO, PEST RESISTANCE ANO FOOO VALUE. 12.021
- HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002
- MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUOOMONAOS. 12.046, 67.109*
- PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004
- PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAD DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*
- PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011
- PLASMIDS IN PLANT PATHOGENIC BACTERIA. 18.012. 26.001*
- SOYBEAN BROWN STEM ROT. 23.008

PLANT-DISEASE-RESISTANCE

- ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010 BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES &
 - MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*
- BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006*
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.035
- CRUCIFER DISEASES. 12.047
- DEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING DISEASE RESISTANCE INTO POTATOES. 11.018
- GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF OURUM AND COMMON WHEAT. 17.025
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH ANO PRODUCTION EFFICIENCY. 12.010, 67.104
- GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004
- GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND OISEASES. **06.003**
- GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011
- GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009
- GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*
- GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001
- GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*
- HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002
- HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113
- ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059
- ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELOIOOGYNE INCOGNITA. 67.053
- MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004,

23 005*

- MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*
- MOLECULAR GENETIC APPROACHES FOR GERMPLASM DEVELOPMENT OF FRUIT AND NUT CROPS. 10.001 PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016
- PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.016
 THE NATURE OF RESISTANCE TO PLANT VIRUSES.
- 12.045

PLANT-DISEASES

- ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010
- BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*
 BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES &
- MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*
- CELLULAR AND MDLECULAR GENETICS FOR CROP IMPROVEMENT. 67.035
- ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*
- GENETIC CONTROL OF LEAF LESION FORMATION IN MAIZE. 14.047
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH
- AND PRODUCTION EFFICIENCY. 12.010
 GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004
- PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004
- PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047
- PLASMIOS IN PLANT PATHOGENIC BACTERIA. 18.012. 26.001*

PLANT-DISTRIBUTION

GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPODIUM). 14.073

PLANT-EMBRYOS

- GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*
- TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

PLANT-ENZYMES

- CELLULAR METABOLISM IN PLANTS. 67.049 CELLULASE GENE EXPRESSION DURING FRUIT OEVELOPMENT. 67.004
- CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.010
- CLONING AND CHARACTERIZATION OF LIGNINASE GENES FROM PHANEROCHAETE CHRYSOSPORIUM. 06.012, 66.011*
- FUNCTION OF PLANT RNA-DEPENDENT RNA POLYMERASES. 12.036
- GENETIC MANIPULATION DF LACTIC DEHYDROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019
- ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015
- ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*
- ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058
- MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012
- ORGANELLE DNA ORGANIZATION AND CYTOPLASMIC

- MALE STERILITY IN PENNISETUM. 20.005 ORGANIZATION AND CONTROL OF THE HYDROLASE GENES IN BARLEY. 18.018
- PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075
- PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028
- RELATIONSHIPS AMONG LIGHT, PHOTDSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

PLANT-EVALUATION

- CYTOPLASMIC FACTORS OF THE PDTATO. 11.012 ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA ANO OTHER EUROPEAN COUNTRIES. 14.080
- MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020
- PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001

PLANT-EXTRACTS

FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034

PLANT-GENETICS

18.003, 67.006*

- ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE
- ELEMENTS. 14.003 ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015
- ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. 67.043
- ANEUPLDID ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007
- BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIDS, AND NATURAL ANTIOXIOANTS IN CORN. 14.014
- BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033 BREEDING, DISEASES AND CULTURE OF DRY PEAS ANO LENTILS. 12.040, 62.006*
- CELL SPECIFIC GENE EXPRESSION DURING LATICIFER DIFFERENTIATION IN POPPY. 67.096
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 18.010, 23.006*, 24.001*, 63.002, 66.002*, 67.026*, 67.027, 67.028, 70.001*
 CELLULAR GENETICS OF CITRUS SPECIES. 09.002
- CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. 62.002
- CELLULASE GENE EXPRESSION DURING FRUIT OEVELOPMENT. 67.004
- CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007
- CHANGES IN WAXY GENE ACTIVITY BY EXCISION OF DS ELEMENT. 14.053
- CHARACTERIZATION OF PLANT GENES ENCODING PHOTOSYNTHETIC MEMBRANE PROTEINS. 67.074
- CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013 CHARACTERIZATION OF THE MAIZE GENOME:
- SEQUENCES CODING FOR SPECIFIC GENES. 67.038
- CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048
- CHILLING AND GENE EXPRESSION IN FRUIT. 12.006 CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.008, 14.010
- CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*
- CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIOIZE TO UNIQUE-SEQUENCE-DNA CLONES. 17.031

- CHROMOSOMAL MAPPING OF GENES CONTROLLING
 TISSUE CULTURE RESPONSE IN WHEAT. 17.010
 CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046
 CLASSICAL GENE MAPPING IN SOYBEAN USING
 MOLECULAR MARKERS. 23.014
- CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. 67.014
- CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006
- CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING DIHYDROFOLATE REDUCTASE. 23.053
- CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIDS AND CYBRIOS OF TOMATO. 12.030
- CONTROL OF GENE EXPRESSION IN POTATO. 11.002 CONTROL OF THE BIOSYNTHESIS OF PROTEINS &
- AMINO AC IOS IN LEGUME SEEOS. 23.046
 CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS
 AND CYTOGENETICS. 14.043
- COSMIO MAPPING OF MITOCHONDRIAL ONA OF MALE FERTILE AND MALE STERILE SORGHUM. 15.002
- CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009
- CRUCIFER DISEASES. 12.047
- CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015
- CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002
- CYTOPLASMIC FACTORS OF THE POTATO. 11.012
 OEVELOPMENT ANO USE OF NEAR ISOGENIC LINES
 FOR GENETIC ANALYSIS OF YIELD AND GENE
 EXPRESSION. 67.018
- OEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002
- OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING DISEASE RESISTANCE INTO POTATOES. 11.018
- DEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. 14.061
- OEVELOPMENTAL GENETICS USING THE ALCHOL OEHYOROGENASE GENE-SYSTEM IN MAIZE. 14.004
- OEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004
- OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015
- DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. **14.056**
- ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEDLING. 14.070
- ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL ANO CHICKPEA USING ISOZYME ANO ONA MARKERS. 12.039
- EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*
- EXPERIMENTAL USE OF ISOZYMES IN APPLIEO PLANT GENETICS RESEARCH. 12.032
- EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*
- EXPRESSION OF MAIZE MITOCHONORIAL GENOME. 14.002
- EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007
- FUNCTION OF PLANT RNA-OEPENOENT RNA POLYMERASES. 12.036
- FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. 67.020
- FURTHER OEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034
- GENE EXPRESSION IN PLANT OEVELOPMENT. 23.033
 GENE EXPRESSION IN PLANTS: A STUDY OF

- POLYADENYLATION IN PLANTS. 20.007

 GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY RECIPROCAL CHROMOSOME SURSTITUTIONS
 - RECIPROCAL CHROMOSOME SUBSTITUTIONS.
 17.024
- GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003*
- GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034, 23.056
- GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007, 15.001*
- GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027
- GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOID WHEAT AND ITS RELATIVES. 67.097
- GENES FOR PHOTOSYNTHESIS IN CORN. **67.058**GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC
 AND QUALITY CHARACTERISTICS OF DURUM AND
 COMMON WHEAT. **17.025**
- GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006 GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005
- GENETIC CONTROL OF LEAF LESION FORMATION IN MAIZE. 14.047
- GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065
- GENETIC CONTROL OF SEMIGAMY AND OERIVATION OF NULLISOMIC COTTON. 21.014, 21.015
- GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018, 07.001, 12.048*, 14.079*, 17.023*, 18.029*
- GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE AND FOOD VALUE. 12.021
- GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004
- GENETIC MANIPULATION OF LACTIC OEHYDROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019
- GENETIC MECHANISMS IN CORN. 14.044
- GENETIC REGULATION OF A SEEO-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*
- GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*
- GENETIC STRUCTURE OF CULTIVATEO SOYBEANS AND THE WILD SOYBEAN (GLYCINE SOJA) GERMPLASM. 23 045
- GENETIC STRUCTURE OF WEEO-CROP POPULATION SYSTEMS (CUCURBITA ANO CHENOPOOIUM).
 14.073
- GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011
- GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015 GENETICS AND CYTOLOGY OF MAIZE. 14.022
- GENETICS AND IMPROVEMENT OF CORN USING
- MOLECULAR ANO TRADITIONAL METHODS. 14.057 GENETICS AND MOLECULAR BIOLOGY OF COTTON
- CYTOPLASMIC MALE STERILITY. 21.003
 GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034,
- 15.004*, 17.018*
 GENETICS OF NITRATE REDUCTION IN BARLEY.
- 67.105
 GENETICS OF SOVREAN WITH SPECIAL EMPHASIS
- GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016
- GENETICS, CYTOGENITCS OF COTTON GERMPLASM. 21.008
- GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*

- GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018
- GENOME EVOLUTION IN BRASSICA. 12.012, 12.013 GENOME ORGANIZATION IN THE CULTIVATED AND WILO SPECIES OF TOMATO. 12.015
- GERMPLASM ENHANCEMENT AND IMPROVEO BREEDING METHOO S IN BARLEY. 18.001
- GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF
- FOOD LÉGUMES. 12.043, 62.007*
 GLIADIN GENES DF CDMMDN WHEAT AND ITS ANCESTORS. 17.011
- GLYPHOSATE OEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*
- HYBRIO VARIEGATION IN PHASEOLUS VULGARIS. 23.038
- ISDLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE FUNCTION AND REGULATION. 14.054
- ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION ANO FUNCTION. 14.059
- ISOLATION AND CHARACTERIZATION OF GENES REGULATEO BY LIGHT. 67.080
- ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003
 ISOLATION AND GENETICS OF GENES INVOLVEO IN
- EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027
- ISDLATION AND TRANSFORMATION OF A WOUNG-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015
- ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*
- ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATDDE MELOIDOGYNE INCDGNITA. 67.053
- ISOLATION OF TRANSPOSON INOUCEO MAIZE MUTANTS OEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001
- ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*
- ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058, 14.063
- ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA ANO OTHER EUROPEAN COUNTRIES. 14.080
- MANIPULATION DF STORAGE PROTEIN STRUCTURE IN
- SOYBEANS. 23.012
 MAPPING THE SYMBIOSIS GENES OF PEA. 67.077 MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060
- MECHANISMS DIRECTING HORMONAL AND OEVELOPMENTAL REGULATION OF GENE EXPRESSIDN IN BARLEY. 18.026
- MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004,
- MITOCHONORIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. 67.030
- MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005
- MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 MOOULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021
- MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA. 67.069
- MOLECULAR AND GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034
- MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATED ATRAZINE TOLERANCE IN CORN. 14.069 MOLECULAR CHARACTERIZATION OF THE SUCROSE

- SYNTHETASE-2 GENE OF MAIZE. 14.011 MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004
- MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003
- MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067
- MOLECULAR GENETIC APPROACHES FOR GERMPLASM DEVELOPMENT OF FRUIT AND NUT CROPS. 10.001
- MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076. 20.016*
- MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017
- MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA DUCH... 10.005
- MOLECULAR MANIPULATION OF GENES. 28.002
- MOLECULAR MAPPING DF GENES IN CORN. 14.040
- MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM OIVERSITY AND SYSTEMATICS. 12.020
- MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021
- MOLECULAR SWITCHES IN PLASTID DIFFERENTIATION. 67.089
- MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047
- MUTANT GENES THAT AFFECT ENDOSPERM DEVELOPMENT IN MAIZE. 14.078
- ORGANELLE ONA ORGANIZATION AND CYTDPLASMIC MALE STERILITY IN PENNISETUM. 20.005
- ORGANIZATION AND CONTROL DF THE HYORDLASE GENES IN BARLEY. 18.018
- ORGANIZATION AND EXPRESSION DF A-AMYLASE GENES IN THE BARLEY GENOME. 62.001
- ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINDSA. 67.092
- ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013
- ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017
- ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077
- PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081, 67.082
- PHYSICAL MAPPING OF THE GENOME DF WHEAT. 17.016
- PHYSIDLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TIDN OF OAT STORAGE PROTEINS. 18.035
- PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. 67.041
- PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075
- PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, ANO OISTRIBUTION. 25.001
- PLANT PROTEINS INVOLVEO IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028
- PLASMIOS IN PLANT PATHOGENIC BACTERIA. 18.012, 26.001*
- PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001, 21.016
- RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*
- REGULATION OF ETHYLENE INOUCEO GENE EXPRESSION OURING FRUIT RIPENING. 67.009
- REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037
- REGULATION OF GENE EXPRESSION IN PLANTS.

14.020

REGULATION OF PLANT GENE EXPRESSION BY ONA METHYLATION. 67.110

REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003

REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSION. 23.001

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*

REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNOS IN HIGHER PLANTS. 67.045

RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN. 23.017

REPEATED ONA SEQUENCES AND CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008

SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013

STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE. 23.035

STRUCTURE AND EXPRESSION OF PLANT GENES ENCOOING CALMOOULIN. 18.014

STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.072

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

STRUCTURE, EXPRESSION AND EVOLUTION OF CYANOBACTERIAL GENES REGULATED OURING NITROGEN FIXATION. 67.084

STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036 STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE

& THEIR USE AS RESEARCH TOOLS. 14.018 SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

SYSTEMS FOR THE MOLECULAR ANALYSIS OF OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007 THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN

ANO COTTON. 14.068, 21.007*
THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001

THE ROLE OF POLYAMINES OURING STRESS-INDUCEO ALTERATIONS IN GENE EXPRESSION. 67.073

TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*, 67.059

TISSUE-SPECIFIC GENE REGULATION IN MAIZE. 14.050

TOWAROS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

TRANSPOSABLE ELEMENTS IN MUTATOR ACTIVITY IN MAIZE. 14.026

TUBULIN GENES OF PLANTS. 67.060

USE OF MOLECULAR MARKERS IN PLANT BREEDING ANO GENETICS. 67.071

USE OF SINGLE COPY CLONEO ONA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028

VARIATION IN LETTUCE COWNY MILCEW. 12.008 VEGETABLE GENETICS. 12.014

WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

PLANT-GROWTH

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS ANO CYTOGENETICS. 14.043 ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS

OEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND OEVELOPMENT. 67.015

MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA OUCH... 10.005

THE ROLE OF POLYAMINES OURING STRESS-INDUCEO ALTERATIONS IN GENE EXPRESSION. 67.073

PLANT-HORMONES

ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003

MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND DEVELOPMENT, 67.015

ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017

REGULATION OF ETHYLENE INOUCEO GENE

EXPRESSION OURING FRUIT RIPENING. 67.009 TISSUE CULTURE AND THE TRANSFER OF

CYTOPLASMIC GENES. 17.027, 18.031*

PLANT-IDENTIFICATION

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035

PLANT-IMPROVEMENT

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010, 67.027

CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002

CYTOPLASMIC FACTORS OF THE POTATO. 11.012 GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.015

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE ANO FOOO VALUE. 12.021

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009

MECHANISMS DIRECTING HORMONAL AND **OEVELOPMENTAL REGULATION OF GENE** EXPRESSION IN BARLEY. 18.026

REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSION. 23.001

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 67.095

RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SOYBEAN. 23.017

STRUCTURE OF GENES INVOLVED IN DIL SYNTHESIS

IN MAIZE. **14.072** Systems for the Molecular analysis of OIFFERENTIAL GENE EXPRESSION IN MAIZE AND OTHER EUKARYOTES. 14.062

PLANT-INJURIES

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

PLANT-INSECT-RESISTANCE

BREEDING, DISEASES AND CULTURE OF DRY PEAS ANO LENTILS. 12.040, 62.006*

CRUCIFER OISEASES. 12.047

GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF OURUM AND COMMON WHEAT. 17.025

GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011

PRESERVATION AND UTILIZATION OF GERMPLASM IN

COTTON. 21.016

PLANT-INTERACTION

CHARACTERIZATION OF THE MI LOCUS CONFERRING RESISTANCE TO MELOIOOGYNE INCOGNITA IN TOMATO. 12.022

PLANT-ION-STRESS

CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048

PLANT-METABOLISM

CELLULAR METABOLISM IN PLANTS. 67.049 CONTROL OF THE BIOSYNTHESIS OF PROTEINS & AMINO AC IOS IN LEGUME SEEOS. 23.046
GENETIC REGULATION OF THE SOYBEAN UREASE

ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*

ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS

OEFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075

REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

THE ROLE OF POLYAMINES OURING STRESS-INDUCEO ALTERATIONS IN GENE EXPRESSION. 67.073 THE THYLAKOIO ENERGY TRANSOUCING ATPASE

COMPLEX. 67.111

PLANT-MICROBIOLOGY

ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027

PLANT-MORPHOLOGY

18.003, 67.006*

CELLULAR GENETICS OF CITRUS SPECIES. 09.002 CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046 CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043

CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034

GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065

GENETIC MECHANISMS IN CORN. 14.044

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. 06.007

GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPOOIUM). 14.073

MAPPING THE SYMBIOSIS GENES OF PEA. 67.077 MITOCHONORIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. 67.030 PLANT-NEMATODES

ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATODE MELOIDOGYNE INCOGNITA. 67.053

PLANT-NUTRITION

GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*
ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA

GENES. 23.055

STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043

PLANT-PATHOGEN

RECOMBINANT ONA APPROACH TO DETECTION AND STRAIN DIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009

PLANT-PATHOGEN-RELATIONS

CRUCIFER DISEASES. 12.047

HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113

PLANT-PATHOGENS

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.013, 26.002*

EXPLOITATION OF RIBOSOMAL ONA TO CONTROL PLANT PATHOGENIC FUNGI. 67.101

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012* PLASMIOS IN PLANT PATHOGENIC BACTERIA

18.012, 26.001*

THE PHYTOPATHOGENIC BACTERIA. 67.052 VARIATION IN LETTUCE COWNY MILOEW. 12.008 PLANT-PATHOLOGY

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010 BIOCHEMISTRY OF PLANT CUTICLE. 67.108

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.035

CRUCIFER DISEASES. 12.047

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELD, PEST RESISTANCE AND FOOD VALUE. 12.021
GENETIC IMPROVEMENT OF POTATO: INCORPORATION

OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034,

15.004*, 17.018* GENETICS OF ASPERGILLUS FLAVUS. 67.036 HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004. 23.005*

MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034

THE PHYTOPATHOGENIC BACTERIA. 67.052 PLANT-PEST-RESISTANCE

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE

AND FOOD VALUE. 12.021
GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

PLANT-PHYSIOLOGY

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

BREEDING, DISEASES AND CULTURE OF DRY PEAS ANO LENTILS. 12.040, 62.006*

CHARACTERIZATION OF NITROGEN FIXATION GENES AND THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057

CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048

CHILLING AND GENE EXPRESSION IN FRUIT. 12.006 DEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING DISEASE RESISTANCE INTO POTATOES. 11.018

OEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

GENETIC MANIPULATION OF LACTIC DEHYDROGENASE ENZYMES IN CEREALS IN SUBPROJECT TITLE. 18.019

GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018*

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*

- MANIPULATION OF STORAGE PROTEIN STRUCTURE IN
- SOYBEANS. 23.012 MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND OEVELOPMENT. 67.015
- MODULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021
- MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004
- ORGANIZATION AND CONTROL OF THE HYDROLASE GENES IN BARLEY. 18.018
- PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081, 67.082
- PHYSIDLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035
- PHYSIOLDGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011
- PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. 67.041
- REGULATION OF ETHYLENE INDUCEO GENE EXPRESSION OURING FRUIT RIPENING. 67.009
- RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023
- REPEATEO ONA SEQUENCES ANO CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008
- SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013
- STRUCTURE DF PHOTOSYNTHETIC MEMBRANES. 67.046 THE ROLE OF POLYAMINES DURING STRESS-INDUCEO ALTERATIONS IN GENE EXPRESSION. 67.073
- THE THYLAKOID ENERGY TRANSOUCING ATPASE CDMPLEX. **67.111**
- TISSUE CULTURE AND THE TRANSFER DF CYTOPLASMIC GENES. 17.027, 18.031*

PLANT-POPULATION

- CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA DNA. 06.011
- GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND DISEASES. 06.003
- GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS DF SPRUCE. 06.007
- SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGD. 20.001

PLANT-PROPAGATION

CELLULAR AND MDLECULAR GENETICS FOR CROP IMPRDVEMENT. 67.027

PLANT-PROTECTION

ISDLATION AND TRANSFORMATION OF A WDUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015

PLANT-PROTEINS

- BIDCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVDLUTION.
- BIDCHEMISTRY OF GENETIC SYSTEMS. 14.033 CELLULAR METABDLISM IN PLANTS. 67.049
- CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHDDS. 17.007
- CHARACTERIZATION DF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TDLERANCE IN PLANTS. 67.048
- CHRDMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.010
- CDNTRDL OF THE BIOSYNTHESIS OF PROTEINS & AMINO AC IOS IN LEGUME SEEOS. 23.046
- OEVELDPMENT AND USE DF NEAR ISDGENIC LINES FOR GENETIC ANALYSIS OF YIELD AND GENE EXPRESSION. 67.018
- OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*
- EVOLUTION OF POLYPLOID WHEATS VIA AMINO ACIO

- SEQUENCING AND ELECTROPHORETIC STUDIES.
- 17.004, 18.005*
 EXPRESSION OF MAIZE MITOCHONORIAL GENOME. 14.002
- GENES FOR PHOTOSYNTHESIS IN CORN. 67.058 MAIZE GRAIN PROTEIN COMPOSITION AND
- OISTRIBUTION AS RELATED TO HARONESS, HANOLING AND PROCESSING. 14.016
- MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*
- MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003
- ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055
- ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013
- PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028
- PLASMIOS IN PLANT PATHOGENIC BACTERIA.
 - 18.012, 26.001*
- REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037
- REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSIDN. 23.001
- STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043
- STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

PLANT-REGENERATION

- CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006
- CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIDS AND CYBRIDS DF TOMATD. 12.030
- GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033 MDLECULAR GENETIC CHANGES ASSOCIATED WITH
- SELECTION IN AGRDNOMIC CROPS. 14.076, 20.016* THE ROLE OF POLYAMINES DURING STRESS-INDUCED
- ALTERATIONS IN GENE EXPRESSION. 67.073 TOWAROS A TRANSPOSON TAGGING SYSTEM IN TDMATO. 12.017

PLANT-REGULATORS

THE PHYTDCHRDME GENE: STRUCTURE, ORGANIZATION ANO EXPRESSIDN. 18.036, 63.007*

PLANT-REPRODUCTION

- GENETIC CONTROL OF SEMIGAMY AND OERIVATION DF NULLISOMIC COTTON. 21.014, 21.015
- GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013
- MOLECULAR CONTROL DF GENE ACTIVITY OURING REPRODUC TIVE OEVELOPMENT IN THE WHEAT PLANT. 17.003
- SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGD. 20.001

PLANT-RESISTANCE

GENETIC IMPROVEMENT DF NORTHWEST TREES. 06.014

PLANT-RESPONSE

CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010

PLANT-STRESS

- CELLULAR METABDLISM IN PLANTS. 67.049 FUNCTION OF PLANT RNA-OEPENOENT RNA POLYMERASES. 12.036
- GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHOOS. 14.057 RECOMBINANT ONA APPRDACHES TO PLANT GENE
- STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*

PLANT-STRESS-RESISTANCE

BREEDING, OISEASES AND CULTURE OF DRY PEAS

ANO LENTILS. 12.040, 62.006*

PLANT-TAXONOMY

GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPOOIUM). 14.073

ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA AND OTHER EUROPEAN COUNTRIES. 14.080

PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.001. 21.016

PLANT-TISSUE

CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

GENE EXPRESSION IN PLANT OEVELOPMENT. 23.033 GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

ISOLATION AND CHARACTERIZATION OF POTATO

TUBERIZATION GENES. 11.003 MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 STRUCTURE. VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036 TISSUE-SPECIFIC GENE REGULATION IN MAIZE.

14.050

PLANT-TOLERANCE

CHILLING AND GENE EXPRESSION IN FRUIT. 12.006 PLANT-TRANSFORMATION

REGULATION OF GENE EXPRESSION BY ABSCISIC ACIO OURING OROUGHT STRESS. 67.016

PLANT-VIRUSES

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING DISEASE RESISTANCE INTO POTATOES. 11.018

GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004

PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047

REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061

THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045

PLANTS

CELLULAR METABOLISM IN PLANTS. 67.049 MITOCHONDRIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005 PLASMID.

BASIC AND APPLIED MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN. 11.008 **PLASMIDS**

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIDIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103

BACTERIAL GENES CODING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.013, 26.002*, 63.002, 66.002*, 67.026*, 70.001*

GENE EXPRESSION IN PLANTS: A STUDY OF POLYADENYLATION IN PLANTS. 20.007

GLYPHOSATE DEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113

ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIDE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027

STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREDII, A NEWLY DESCRIBEO SPECIES OF SOYBEAN RHIZ. 23.051, 66.009* THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN AND COTTON. 14.068, 21.007*

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. 67.054

PLASMIDS-(BACTERIAL)

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*

MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUOOMONAS SPECIES. 67.034

PLASMIDS IN PLANT PATHOGENIC BACTERIA. 18.012. 26.001*

THE PHYTOPATHOGENIC BACTERIA. 67.052

PLASTIDS

GENES FOR PHOTOSYNTHESIS IN CORN. 67.058 HYBRIO VARIEGATION IN PHASEOLUS VULGARIS. 23.038

MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. 67.089 PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.082

PLEUROTUS-SPP

GERMPLASM ENHANCEMENT AND CULTURE OF EDIBLE MUSHROOMS. 12.038

PLOIDY

CYTOGENETIC STUDIES OF HARDWOOD AND CONIFEROUS FOREST TREES. 06.016

POLLEN-GENE-EXPRESSION.

TRANSPOSON MUTAGENESIS IN TOMATO. 12.016 POLY-HAPLOIDS

GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014, 21.015

POLYAMINES

THE ROLE OF POLYAMINES OURING STRESS-INDUCEO ALTERATIONS IN GENE EXPRESSION. 67.073

POLYGALACTURONASE

MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUOOMONAS SPECIES. 67.034

POLYMORPHISM

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.010

CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. 06.011

CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIDIZE TO UNIQUE-SEQUENCE-DNA CLONES. 17.031

CHROMOSOME SEGMENTAL EFFECTS IN CORN. 14.046 CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014

CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIDS AND CYBRIOS OF TOMATO. 12.030

CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS. 14.032, 23.024*,

30.001*, 31.002*
CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015

CYTOGENETIC MANIPULATIONS FOR ALFALFA IMPROVEMENT. 20.002

DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND DNA MARKERS. 12.039

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 17.023*, 18.029*

GENETIC STRUCTURE OF CULTIVATED SOYBEANS AND THE WILO SOYBEAN (GLYCINE SOJA) GERMPLASM.

GENETICS AND BREEDING OF COOL SEASON CROPS.

12.011

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018*

HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002

INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LOOGEPOLE AND JACK PINES, **06.004**

MAPPING THE SYMBIOSIS GENES OF PEA. 67.077
MOLECULAR CHARACTERIZATION OF THE SUCROSE
SYNTHETASE-2 GENE OF MAIZE. 14.011

MOLECULAR GENETIC APPROACHES FOR GERMPLASM OEVELOPMENT OF FRUIT AND NUT CROPS. 10.001

MOLECULAR MAPPING OF GENES IN CORN. 14.040
MOLECULAR MARKERS FOR EVALUATION OF SWEET
POTATO GERMPLASM OIVERSITY ANO
SYSTEMATICS. 12.020

MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021

PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016

STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036

USE OF SINGLE COPY CLONEO ONA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028
VARIATION IN LETTUCE ODWNY MILOEW. 12.008

POLYMORPHISMS

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND DNA MARKERS. 12.041

GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

POLYMORPHISMS-(RFLP)

ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.030

POLYPEPTIDES

CELL SPECIFIC GENE EXPRESSION OURING
LATICIFER OIFFERENTIATION IN POPPY. 67.096
GENES FOR PHOTOSYNTHESIS IN CORN. 67.058
PLANT GENE REGULATION OURING NITROGEN
ASSIMILATION. 67.075

THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION. 18.036, 63.007*

POLYPHENOL-OXIDASE

EXPRESSION OF MELANIN IN PLANTS - MONITORING GENE EXPRESSION. 12.002, 13.001, 67.001

POLYPHENOLS

GENETIC MECHANISMS IN CORN. 14.044 POLYPLOIDS

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015
GENOME EVOLUTION IN BRASSICA. 12.012
GERMPLASM ENHANCEMENT AND IMPROVED BREEDING

METHOO S IN BARLEY. 18.001

MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA OUCH...
10.005

POLYSACCHARIDES

ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIDE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027

POLYUNSATURATED-FATTY-ACIDS

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

POPCORN

METHODS OF CONTROLLING CORN INSECTS. 14.024

MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*

POPULATION-DYNAMICS

ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

POPULATION-GENETICS

18.003, 67.006*

CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS. 14.032, 23.024*, 30.001*. 31.002*

GENE EXPRESSION IN PLANTS: A STUDY OF POLYAOENYLATION IN PLANTS. 20.007

GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS. 14.057

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018*

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE FUNCTION AND REGULATION. 14.054

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN SOUTHERN DREGON. 06.013

PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, AND OISTRIBUTION. 25.001

POST-HARVEST

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

POTATO

MOLECULAR GENETICS OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.005

RECOMBINANT DNA APPROACH TO OETECTION ANO STRAIN OIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009

SYSTEMATICS OF POTATOES AND THEIR WILD RELATIVES (SOLANUM SECT. PETOTA). 11.017

POTATO-RING-ROT

RECOMBINANT ONA APPROACH TO OETECTION ANO STRAIN OIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009

POTATO-VIRUS-X

MOLECULAR GENETICS OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.005

POTATO,

BASIC AND APPLIED MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN. 11.008

POTATOES

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010 CONTROL OF GENE EXPRESSION IN POTATO. 11.002 CYTOPLASMIC FACTORS OF THE POTATO. 11.012 DEVELOPMENT OF A ONA HYBRIOIZATION ASSAY FOR OFTECTION OF THE RING ROT PATHOGEN. 11.007

OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING DISEASE RESISTANCE INTO POTATOES. 11.018

OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015

EXPLORATORY RESEARCH ON THE CYTOGENETICS OF SOLANACEOUS CROPS. 11.001

GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004

ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*, 67.095

POTY-VIRUSES

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. 67.054

PROBES

CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. 06.011 CHROMATIN STRUCTURE AND GENE EXPRESSION IN

MAIZE. 14.010

CONTROL OF GENE EXPRESSION IN POTATO. 11.002 CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). 14.015

DEVELOPMENT OF A DNA HYBRIOIZATION ASSAY FOR OETECTION OF THE RING ROT PATHOGEN. 11.007 DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING

COMPLEX GENES. 23.004

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015

GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007, 15.001*

MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*

MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021

ORGANIZATION AND EXPRESSION OF LEGHEMOGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINOSA. 67.092

THE NATURE OF RESISTANCE TO PLANT VIRUSES.
12.045

TUBULIN GENES OF PLANTS. 67.060

USE OF SINGLE COPY CLONEO ONA SEQUENCES AS GENETIC MARKERS IN BARLEY. 18.028

VARIATION IN LETTUCE OOWNY MILOEW. 12.008 PROGENY

OISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056
PROGENY-SELECTION

MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*

TOWAROS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

PROGENY-TESTING

GENETIC CONTROL OF SEMIGAMY; ANO OERIVATION OF NULLISOMIC COTTON. 21.013

PROMOTERS-(GENETICS)

CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006

OEFINING AND MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011

MOLECULAR GENETIC APPROACHES FOR GERMPLASM OEVELOPMENT OF FRUIT AND NUT CROPS. 10.001 ORGANIZATION AND CONTROL OF THE HYDROLASE

GENES IN BARLEY. 18.018
REGULATION OF PLANT GENE EXPRESSION BY ONA

METHYLATION. 67.110

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

PROPIONIBACTERIUM

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOODS. 14.025, 31.001*

PROPIONIC-ACID

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOODS. 14.025, 31.001*

PROTEIN-ANALYSIS

GENES FOR PHOTOSYNTHESIS IN CORN. 67.058

PROTEIN-BINDING

DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004

ISOLATION OF GENES THAT ENCODE ONA BINOING PROTEINS. 67.066

MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND OEVELOPMENT. **67.015**

PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028

REGULATION OF SOYBEAN SEEO PROTEIN GENE EXPRESSION. 23.001

STRUCTURE AND EXPRESSION OF PLANT GENES ENCODING CALMOOULIN. 18.014

PROTEIN-BIOSYNTHESIS

ANALYSIS OF THE OIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005

MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004

MUTANT GENES THAT AFFECT ENOOSPERM OEVELOPMENT IN MAIZE. 14.078

REGULATION OF GENE EXPRESSION IN PLANTS. 14.020

PROTEIN-CHARACTERIZATION

CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048

CHROMATIN STRUCTURE AND GENE EXPRESSION IN MAIZE. 14.010

PROTEIN-COMPOSITION

MAIZE GRAIN PROTEIN COMPOSITION ANO OISTRIBUTION AS RELATED TO HARONESS, HANOLING AND PROCESSING. 14.016

PROTEIN-CONTENT

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIOANTS IN CORN. 14.014

OEVELOPMENT AND USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELO AND GENE EXPRESSION. **67.018**

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015

PROTEIN-DNA-INTERACTIONS

GENE EXPRESSION IN PLANT OEVELOPMENT. 23.033 PROTEIN-IDENTIFICATION

REGULATION OF SOYBEAN SEEO PROTEIN GENE EXPRESSION. 23.001

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. **67.044**

PROTEIN-ISOLATES

ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATOOE MELOIOOGYNE INCOGNITA. **67.053 PROTEIN-LEVELS**

GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005

PROTEIN-METABOLISM

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075

PROTEIN-PURIFICATION

CHARACTERIZATION OF NITROGEN FIXATION GENES ANO THEIR PRODUCTS FROM AZOTOBACTER VINELANOII. 23.057

MOLECULAR BASIS OF GSH-S TRANSTERASE MEDIATEO ATRAZINE TOLERANCE IN CORN. 14.069

MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046 THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007

PROTEIN-QUALITY
BIOCHEMICAL CHARACTERIZATION OF STORAGE

PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007

CONTROL OF THE BIOSYNTHESIS OF PROTEINS &

AMINO AC IOS IN LEGUME SEEDS. 23.046
GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC
AND QUALITY CHARACTERISTICS OF OURUM AND
COMMON WHEAT. 17.025

GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005

WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

PROTEIN-SEQUENCE

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING DIHYDROFOLATE REDUCTASE. 23.053

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE FUNCTION AND REGULATION. 14.054

ISOLATION ANO CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE STRUCTURE, REGULATION ANO FUNCTION. 14.059

PROTEIN-STRUCTURE

ANALYSIS OF THE OIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033 EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING ANO ELECTROPHORETIC STUDIES. 17.004, 18.005*

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012

SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013

THE EVOLUTION OF GENE LOCI IN PLANTS. 67.007

PROTEIN-SYNTHESIS CHARACTERIZATION OF THE ROLE OF A STRESS

PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048

CONTROL OF THE BIOSYNTHESIS OF PROTEINS & AMINO AC IOS IN LEGUME SEEOS. 23.046

DEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING OISEASE RESISTANCE INTO POTATOES. 11.018

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

GENETICS AND MOLECULAR BIOLOGY OF COTTON
CYTOPLASMIC MALE STERILITY. 21.003

MAIZE GRAIN PROTEIN COMPOSITION AND DISTRIBUTION AS RELATED TO HARDNESS, HANDLING AND PROCESSING. 14.016

MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004

MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003

ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055

PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035

PLANT GENE REGULATION OURING NITROGEN ASSIMILATION. 67.075

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*, 67.095

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

PROTEINASES

DIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015
STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*,

20.014*, 26.006*, 63.006*

PROTEINS

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

CHARACTERIZATION OF PLANT GENES ENCOOING
PHOTOSYNTHETIC MEMBRANE PROTEINS. 67.074

OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING OISEASE RESISTANCE INTO POTATOES. 11.018

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004. 18.005*

17.004, 18.005*
FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. 67.020

GLIAOIN GENES OF COMMON WHEAT ANO ITS ANCESTORS. 17.011

ISOLATION OF GENES THAT ENCODE DNA BINDING PROTEINS. **67.066**

MECHANISMS DIRECTING HORMONAL AND OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026

MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034

MUTANT GENES THAT AFFECT ENOOSPERM OEVELOPMENT IN MAIZE. 14.078

PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*

REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061

RFLP METHODOLOGY FOR RELATEONESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS.

17.006, 67.021* PROTOPLAST-FUSION

GENETICS OF ASPERGILLUS FLAVUS. 67.036
MAPPING, TRANSFER, RECOMBINATION AND
EXPRESSION OF ORGANELLE AND NUCLEAR GENES.
16.004, 17.020*, 23.031*, 67.056*

PROTOPLASTS

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*, 67.028

CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. **62.002**

CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIOS AND CYBRIDS OF TOMATO. 12.030

DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS. 06.008

GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOODS. 14.025, 31.001*

MITOCHONDRIAL DNA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. 67.030

TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. 67.054

PSEUDOMONAS

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.013, 26.002*

MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUCOMONAS SPECIES. 67.034

PSEUDOMONAS-SOLANACEARUM

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. **67.035**HOST-PATHOGEN RECOGNITION AND DISEASE

RESISTANCE IN PLANTS. 67.113

MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034

PSEUDOMONAS-SYRINGAE

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.035

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS VULGARIS L.) FOR YIELO, PEST RESISTANCE ANO FOOO VALUE. 12.021

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*

MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034

MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*

PLASMIOS IN PLANT PATHOGENIC BACTERIA. 18.012, 26.001*

PSEUDOMONAS-TABACI

MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*

PLASMIOS IN PLANT PATHOGENIC BACTERIA. 18.012, 26.001* PSEUDOTSUGA-MENZIESII

GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF DOUGLAS-FIR IN SOUTHERN OREGON. 06.013

PURIFICATION

MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067 **PYRETHROIDS**

POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS. 21.004

QUALITY-MAINTENANCE

MAIZE GRAIN PROTEIN COMPOSITION AND OISTRIBUTION AS RELATED TO HARONESS. HANDLING AND PROCESSING. 14.016

QUANTITATIVE

CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE. 27.003

QUANTITATIVE-TRAIT-LOCI

EVALUATION OF GENETIC VARIABILITY IN ECHINACEA. 25.005, 28.003*

QUANTITATIVE-TRAITS

ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.030

QUANTITATIVE-TRAITS.

ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.029

CLONING CULTIVAR SPECIFICITY GENES FROM MAGNAPORTHE GRISEA. 16.010

RACES-(GENETICS)

HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060

MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

RADIANT-ENERGY

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046 RADIO-LABELING

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010 EUROPEAN CORN BORER PHEROMONE STRAINS: FATE

OF HYBRIOIZING POPULATIONS. 14.051 VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. 67.054

RADIOACTIVE-TRACERS

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

RADISHES

CRUCIFER DISEASES. 12.047

EXPRESSION OF EMBRYO-SPECIFIC GENES DURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002. 67.042*

RAPESEED

CRUCIFER DISEASES. 12.047 GENETIC ENGINEERING OF OILSEED SPECIES TO IMPROVE OIL QUALITY. 23.020, 25.003*

REGULATION OF GRASSHOPPER REPRODUCTION BY JUVENILE HORMONE. 67.023

RECIPROCAL-CROSSING

CYTOPLASMIC FACTORS OF THE POTATO. 11.012 RECOMBINANT

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

RECOMBINANT-DNA

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. 67.043

BIOCHEMISTRY OF PLANT CUTICLE. 67.108 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.013, 26.002*, 67.028

CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007

CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN, 17.012

CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013

CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES COOING FOR SPECIFIC GENES. 67.038

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING DIHYDROFOLATE REDUCTASE. 23.053

OFFINING AND MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*

OEVELOPMENT OF A ONA HYBRIDIZATION ASSAY FOR DETECTION OF THE RING ROT PATHOGEN. 11.007

DEVELOPMENTAL GENETICS USING THE ALCHOL DEHYDROGENASE GENE-SYSTEM IN MAIZE. 14.004

EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002. 67.042*

GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SORGHUM. 14.007,

GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027

GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. **17.005**

GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVED AGRONOMIC PROPERTIES. 23.039 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018, 12.048*, 14.079*

GENETICS OF NITROGEN FIXATION IN AZOTOBACTER VINELANDII. 23.048, 23.049, 66.006*, 66 007*

GLIADIN GENES DF COMMON WHEAT AND ITS

ANCESTORS. 17.011
IMPRDVING THE EFFICACY OF BACULOVIRUS PESTICIOES BY RECDMBINANT ONA TECHNOLOGY. 65.002, 67.031*

ISDLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM, 23.027

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005

MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE OEVELOPMENT IN THE WHEAT PLANT. 17.003

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047

PATHO-PHYSIOLOGY DF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

RECOMBINANT ONA APPROACH TO OETECTION AND STRAIN OIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009

RECDMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*

REGULATION OF GENE EXPRESSION IN PLANTS. 14.020

STRUCTURE AND EXPRESSION OF PLANT GENES ENCOOING CALMDOULIN. 18.014

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001

THE PHYTOPATHDGENIC BACTERIA. 67.052 TISSUE CULTURE AND THE TRANSFER OF

CYTOPLASMIC GENES. 17.027, 18.031* TRANSPOSABLE ELEMENTS IN MUTATOR ACTIVITY IN MAIZE. 14.026

WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

RECOMBINANT-DNA,

BASIC AND APPLIED MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN. 11.008

RECOMBINANT-INBRED-LINES

BARLEY BREEDING AND GENETICS. 18.027 MOLECULAR MARKERS FOR BRASSICA CAMPESTRIS CHROMDSDMES. 12.026

RECOMBINANT-RNA

MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA OUCH.. 10.005

RECOMBINATION

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

ORGANIZATION OF THE R CHROMOSOME REGION IN

MAIZE. 14.077
STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PROOUCTS. 67.044

RECOMBINATION-MAPPING

GENETICS DF BIOTYPES IN THE HESSIAN FLY (MAYETIDLA OESTRUCTOR). 17.014

RECURRENT-SELECTION

GENETICS AND IMPROVEMENT OF CORN USING MOLECULAR AND TRADITIONAL METHODS. 14.057 GERMPLASM ENHANCEMENT AND IMPROVED BREEDING METHOD S IN BARLEY. 18.001

RED-BEANS

GENETIC IMPROVEMENT OF BEANS (PHASEOLUS

VULGARIS L.) FOR YIELD, PEST RESISTANCE ANO FD00 VALUE. 12.021

RED-LIGHT

ISOLATION AND CHARACTERIZATION OF GENES REGULATEO BY LIGHT. 67.080

REDUCTASES

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH ANO PRODUCTION EFFICIENCY. 67.104

REDUCTION-(CHEMISTRY)

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CD(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

REGENERATION

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*,

CELLULAR GENETICS OF CITRUS SPECIES. 09.002 TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

REGULATION

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*

REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF AROMATIC COMPOUNDS IN HIGHER PLANTS. 67.045

REGULATORS

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE FUNCTION AND REGULATION. 14.054

RELATIONSHIPS

SYSTEMATICS DF POTATOES AND THEIR WILD RELATIVES (SOLANUM SECT. PETOTA). 11.017

REPORTER-GENE

THE MDLECULAR BASIS OF BLACK ROT OF CRUCIFERS. 12.001

REPRODUCTION.

REGULATION DF GRASSHOPPER REPRODUCTION BY JUVENILE HDRMDNE. 67.023

RESEARCH-PROGRAMS

GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014

RESISTANCE

MDLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO. 65.001, 67.012*

RESISTANCE-MANAGEMENT

POPULATION STRUCTURE AND THE EVOLUTION OF RESISTANCE IN HELIOTHIS VIRESCENS. 21.004

RESISTANT

RFLP METHOOOLOGY FOR RELATEONESS AMONG SMALL GRAINCEREAL ACCESSIDNS. 18.002

RESTRICTION

GENETIC MAPPING OF PEAS, LENTILS AND CHICKPEAS. 12.042

RESTRICTION-ENZYMES

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIOIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103

ANALYSIS OF THE GENETIC ARRANGEMENT OF ZEA MAYS MTONA. 67.043

BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 18.013, 26.002*

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTDPLASMIC MANIPULATIONS. 17.009

CYTDGENETIC MANIPULATIONS FDR ALFALFA

IMPROVEMENT. 20.002
DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT DF WDDDY PLANTS. 06.008

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISDZYME AND DNA

MARKERS. 12.039

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034

GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027

GENETIC SELECTION-MANIPULATION TO ENHANCE PREDATOR AND PARASITE EFFECTIVENESS. 14.045, 17.022*, 18.023*, 20.009*, 21.005*, 23.040*

HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002

INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST ONA POLYMORPHISMS IN LODGEPOLE AND JACK PINES. 06.004

AND JACK PINES. 06.004
MITOCHONDRIAL ONA VARIATION IN SUBPOPULATIONS
OF SOMATIC HYBRIO CELLS OF GRASSES. 67.030

MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020

MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021

MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. **67.089**

ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. **18.017**

REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003

THE PHYCOCYANIN GENES OF AGMENELLUM QUADRUPLICATUM. 67.088

RESTRICTION-FRAGMENT-LENGTH

ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.030

RESTRICTION-FRAGMENT-LENGTH-PO

OEVELOPMENT OF NEW AND IMPROVEO CROPS FOR WATER CONSERVATION IN ARIO LANDS. **25.004**

GENETICS OF BIOTYPES IN THE HESSIAN FLY (MAYETIOLA DESTRUCTOR). 17.014

ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.029

RESTRICTION-FRAGMENTS

ESTABLISHMENT OF A GENE LINKAGE MAP IN LENTIL AND CHICKPEA USING ISOZYME AND DNA MARKERS. 12.041

RESTRICTION-LENGTH

EVALUATION OF GENETIC VARIABILITY IN ECHINACEA. 25.005, 28.003*

RESTRICTION-POLYMORPHISMS

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN SORGHUM. 15.009

REVERTANTS

GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

ORGANELLE ONA ORGANIZATION AND CYTOPLASMIC

MALE STERILITY IN PENNISETUM. 20.005

RFLP

BARLEY BREEDING AND GENETICS. 18.027 CONSTRUCTION OF A SATURATED GENETIC LINKAGE MAP FOR LOBIOLLY PINE. 06.002

MAP FOR LOBLOLLY PINE. **06.002**RECOMBINANT DNA APPROACH TO DETECTION AND STRAIN DIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. **11.009**

RFLP AND MOLECULAR ANALYSIS OF ROOT KNOT NEMATODES, NEMATODE INFECTED PLANTS AND PEACHES. 10.009, 23.054*

RFLP METHOOOLOGY FOR RELATEDNESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

SYSTEMATICS OF POTATOES AND THEIR WILD RELATIVES (SOLANUM SECT. PETOTA). 11.017 RFLP-ANALYSIS,

MOLECULAR MARKERS FOR BRASSICA CAMPESTRIS CHROMOSOMES. 12.026

RFLP

BASIC AND APPLIED MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN. 11.008

RFLPS

CHROMOSOMAL LOCALIZATION AND INTRACHROMOSOMAL MAPPING OF WHEAT RFLP LOCI. 17.032

MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO. 65.001.

67.012*

TAGGING PLANT GENES WITH TIGHTLY-LINKED RFLP MARKERS. 12.035

RFLPS,

GENETIC/MOLECULAR CHARACTERIZATION OF THE MUTATOR-RELATED CY TRANSPOSABLE ELEMENT SYSTEM OF MAIZE. 14.031

RHIZOBIUM

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*, 63.002, 66.002*, 67.026*, 70.001* MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022

STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREDII, A NEWLY DESCRIBED SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

RHIZOBIUM-JAPONICUM

GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVED AGRONOMIC PROPERTIES. 23.039 ISOLATION AND GENETICS OF GENES INVOLVED IN

ISOLATION AND GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIDE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027

MODULATION AND NITROGEN FIXATION OF PRC R. JAPONICUM. 23.021

STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREDII, A NEWLY DESCRIBED SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

RHIZOBIUM-MELILOTI

AMINO ACIO METABOLISM AND THE RHIZOBIUM-LEGUME SYMBIOSIS. 66.014, 67.107*

RHODOPSEUDOMONAS-SPHAEROIDES

BACTERIAL GENES CODING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. 67.098

RIBONUCLEASE

THE REPLICATION OF CHLOROPLAST ONA IN CHLAMYOOMONAS REINHAROII. **67.057**

RIBOSOMAL - DNA

GENETICS OF BIOTYPES IN THE HESSIAN FLY (MAYETIOLA DESTRUCTOR). 17.014
RESEARCH ON THE INTRASPECIFIC VARIATION IN CENOCOCCUM GEOPHILUM FR.. 67.076

RIBOSOMES

CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA DNA. **06.011**STRUCTURE. VARIATION AND INHERITANCE OF

RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036

RIBULOSE-BIPHOSPHATE

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

RICE

CLONING CULTIVAR SPECIFICITY GENES FROM MAGNAPORTHE GRISEA. 16.010

CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006

GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003*

HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*
MOLECULAR GENETIC EVALUATION OF PLANT

```
GERMPLASM RESOURCES. 67.017
  THE MOLECULAR BIOLOGY OF THE RICE
     ALPHA-AMYLASE GENES. 16.001
RING-ROT-(POTATOES)
  ALTERNATIVE APPROACHES TO POTATO RING ROT
     RESEARCH AND VARIETAL IMPROVEMENT. 11.010
  GEVELOPMENT OF A ONA HYBRIOIZATION ASSAY FOR
     DETECTION OF THE RING ROT PATHOGEN. 11.007
RIPENING
  CELLULASE GENE EXPRESSION OURING FRUIT
     OEVELOPMENT. 67.004
  REGULATION OF ETHYLENE INDUCED GENE
     EXPRESSION OURING FRUIT RIPENING. 67.009
  ANALYSIS OF SEEO GLOBULIN GENES AND THEIR
     EXPRESSION IN CEREALS. 18.016
  CELL SPECIFIC GENE EXPRESSION OURING
     LATICIFER OIFFERENTIATION IN POPPY. 67.096
  CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA
    METHOOS. 17.007
  EXPLOITATION OF RIBOSOMAL ONA TO CONTROL
     PLANT PATHOGENIC FUNGI. 67.101
  FUNCTION OF PLANT RNA-OEPENOENT RNA
     POLYMERASES. 12.036
  GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT
     PROTEINS. 17.005
  ISOLATION AND CHARACTERIZATION OF CORN GENES
     TO ELUCIDATE GENE STRUCTURE, REGULATION
     AND FUNCTION. 14.059
  ISOLATIONS, MOLECULAR CHARACTERIZATIONS &
     CLONING OF GENES FROM MUTANTS REGULATING
     LYSINE IN CEREALS. 16.003, 17.019*,
     23.030*, 26.003*
  MECHANISMS DIRECTING HORMONAL AND
     OEVELOPMENTAL REGULATION OF GENE
     EXPRESSION IN BARLEY. 18.026
  MOLECULAR AND GENETIC STUDIES OF NITRATE
     REDUCTASE IN BARLEY. 18.034
  MOLECULAR MANIPULATION OF GENES. 28,002
  MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE:
     LEGUMINOSAE). 23.047
  ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA
     GENES. 23.055
  PHOTOREGULATED GENE EXPRESSION IN
     CHLOROPLASTS. 67.082
  PLANT GENE REGULATION OURING NITROGEN
     ASSIMILATION. 67.075
  STRUCTURE AND EXPRESSION OF PLANT GENES
     ENCOOING CALMODULIN. 18.014
  STRUCTURE AND EXPRESSION OF SOYBEAN LEAF
     STORAGE PROTEIN GENES. 23.043
  STRUCTURE, VARIATION AND INHERITANCE OF
     RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036
  THE PHYCOCYANIN GENES OF AGMENELLUM
     QUADRUPLICATUM. 67.088
  THE REPLICATION OF CHLOROPLAST DNA IN
     CHLAMYOOMONAS REINHAROII. 67.057
RNA-HYBRIDIZATION
  MOLECULAR SWITCHES IN PLASTIO
     DIFFERENTIATION. 67.089
  ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA
     GENES. 23.055
RNA-M
  A COMPARATIVE STUDY OF N(2)-FIXING GENES IN
```

CLOSTRIOIUM PASTEURIANUM AND OTHER

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF DAT

CHILLING AND GENE EXPRESSION IN FRUIT. 12.006

INCOR- PORATING DISEASE RESISTANCE INTO

INHIBITOR GENES IN PLANT TISSUES. 11.015

DEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR

POTATOES. 11.018
OIFFERENTIAL EXPRESSION OF PROTEINASE

MICROORGANISMS. 67.103

STORAGE PROTEINS. 18.015

0

0

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007 GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033 GENE EXPRESSION IN PLANTS: A STUDY OF POLYAGENYLATION IN PLANTS. 20.007 GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 67.104 GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004 ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003 ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015 MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056* MOLECULAR CLONING OF THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN. 14.067 ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. 65.004, 67.040* REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 11.014, 67.094*, 67.095 WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021* RNA-POLYMERASE FUNCTION OF PLANT RNA-OEPENOENT RNA POLYMERASES. 12.036 REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061 RNA-R RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085* RNA-SEQUENCES GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 67.104 ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003 MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005 RNA-SYNTHESIS THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001 RNA-VIRUSES PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. **67.047** ROBERTSON'S-MUTATOR ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.030 ROBERTSON'S-MUTATOR. ISOLATION OF GENES FOR QUANTITATIVE INHERITANCE IN MAIZE. 14.029 ROOT-CROPS ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010 ROOT-KNOT-NEMATODES MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO. 65.001. 67.012* ROOT-ROT BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006* PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011 ROOT-ROT-(COTTON) ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010* ROOTS

ONA METHYLATION AND CONTROL OF GENE

SEEDLING. 14.070

EXPRESSION IN SHOOT AND ROOT OF MAIZE

EFFICIENCY OF NITROGEN FIXATION. 23.025,

66.003*

RUBBER

MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004

RUST

EXPLOITATION OF RIBOSOMAL ONA TO CONTROL PLANT PATHOGENIC FUNGI. 67.101

S-GENE

STRUCTURE AND FUNCTION OF
SELF-INCOMPATIBILITY GENES: A
BIOTECHNOLOGICAL APPROACH. 11.013, 67.090*
S-LOCUS,

MOLECULAR ANALYSIS OF THE S LOCUS OF BRASSICA. **67.068**

SACCHAROMYCES-CEREVISIAE

CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013 COOING ANO REGULATION OF MITOCHONORIAL GENES. 14.052

ISOLATION OF GENES THAT ENCODE ONA BINOING PROTEINS. 67.066

SALT

CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048

SALT-TOLERANCE

MOLECULAR GENETIC APPROACHES FOR GERMPLASM
OEVELOPMENT OF FRUIT AND NUT CROPS. 10.001
TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA

SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

SCENEDESMUS

BIOCHEMICAL ANO OEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

SCHIZOPHYLLUM

EXPLOITATION OF RIBOSOMAL ONA TO CONTROL PLANT PATHOGENIC FUNGI. **67.101**

SCHIZOPHYLLUM-COMMUNE

ISOLATING ANO CHARACTERIZING A-BETA MATING-TYPE ALLELES OF SCHIZOPHYLLUM COMMUNE.. 66.013, 67.102*

SCI FROTTA

ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

SCLEROTINIA

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 17.023*, 18.029*

SCREENING

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

CONTROL OF GENE EXPRESSION IN POTATO. 11.002 EXPERIMENTAL USE OF ISOZYMES IN APPLIEO PLANT GENETICS RESEARCH. 12.032

MOLECULAR AND GENETIC STUDIES OF NITRATE REDUCTASE IN BARLEY. 18.034

STRUCTURE AND EXPRESSION OF PLANT GENES ENCOOING CALMODULIN. 18.014

VEGETABLE GENETICS. 12.014

SEED

RFLP METHOOOLOGY FOR RELATEONESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

ROLE OF LOW MOLECULAR WEIGHT HEAT SHOCK PROTEINS IN PLANT DEVELOPMENT. **67.002**

SEED-CERTIFICATION

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 06.018, 12.048*. 14.079*

12.048*, 14.079*
PATTERNS OF GENETIC VARIATION AMONG

BREEOING/SEEO ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. 06.013

SEED-DEVELOPMENT

ANALYSIS OF SEEO GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015
MOLECULAR CONTROL OF GENE ACTIVITY OURING

MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE OEVELOPMENT IN THE WHEAT PLANT. 17.003

REGULATION OF FATTY ACID SYNTHESIS PROTEINS IN SOYBEAN. 23.037

REGULATION OF GENE EXPRESSION IN PLANTS. 14.020

SEED-DORMANCY

ISOLATION OF TRANSPOSON INDUCEO MAIZE MUTANTS OFFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001

SEED-GERMINATION

EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

SEED-INCREASE

CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014

PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001

SEED-MATURATION

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.005

SEED-OIL-BIOSYNTHESIS,

STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS IN MAIZE. 14.005

SEED-OILS,

STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS IN MAIZE. 14.005

SEED-PHYSIOLOGY

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

SEED-PRODUCTION

MECHANISM OF ACTION OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH ANO OEVELOPMENT. **67.015**

SEED-PROTEINS

ANALYSIS OF SEED GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016

ANALYSIS OF THE OIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY RECIPROCAL CHROMOSOME SUBSTITUTIONS. 17.024

GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

GENETIC STRUCTURE OF CULTIVATEO SOYBEANS ANO THE WILD SOYBEAN (GLYCINE SOUA) GERMPLASM. 23.045

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047

REGULATION OF GENE EXPRESSION IN PLANTS.
14.020

REGULATION OF SOYBEAN SEED PROTEIN GENE EXPRESSION. 23.001

SEED-STOCK-MAINTENANCE

PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OCCUMENTATION, MAINTENANCE, AND DISTRIBUTION. 25.001

SEED-STOCKS

GENETIC STRUCTURE OF WEED-CROP POPULATION SYSTEMS (CUCURBITA AND CHENOPODIUM). 14.073

MEASURING THE GENETIC DIVERSITY OF CULTIVATED PLANTS USING ISOZYME FREQUENCIES. 14.060

SEEDLINGS

CLONING, EXPRESSION AND MUTAGENESIS OF SOYBEAN SEEDLING OIHYOROFOLATE REDUCTASE. 23 053

SEEDS

ANALYSIS OF THE OIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. **62.002** CONTROL OF THE BIOSYNTHESIS OF PROTEINS &

AMINO AC IOS IN LEGUME SEEOS. 23.046
MECHANISM OF ACTION OF PLANT GROWTH

SUBSTANCES IN PLANT GROWTH AND DEVELOPMENT. 67.015

PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. 06.013

REGULATION OF FATTY ACIO SYNTHESIS PROTEINS IN SOYBEAN. 23.037

REGULATION OF GENE EXPRESSION IN PLANTS. 14.020

REGULATION OF SOYBEAN SEEO PROTEIN GENE EXPRESSION. 23.001

SELECTION

CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS. 14.032, 23.024*, 30.001*, 31.002*

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

ISOZYME LOCI AS MARKERS FOR LOCATING ANO MANIPULATING QUANTITATIVE TRAIT LOCI. 14.063

MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021

VEGETABLE GENETICS. 12.014

SELECTION-SYSTEMS

•)

ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI. 14.058

SELF-INCOMPATIBILITY

MOLECULAR ANALYSIS OF S-LOCUS EXPRESSION IN ANTHERS OF BRASSICA. **67.069**

MOLECULAR GENETIC ANALYSIS OF THE BRASSICA S LOCUS. 67.067

STRUCTURE AND FUNCTION OF SELF-INCOMPATIBILITY GENES: A BIOTECHNOLOGICAL APPROACH. 11.013, 67.090*

SELF-INCOMPATIBILITY,
MOLECULAR ANALYSIS OF THE S LOCUS OF
BRASSICA. 67.068

SELF-POLLINATION

OEVELOPMENT ANO USE OF NEAR ISOGENIC LINES FOR GENETIC ANALYSIS OF YIELO ANO GENE EXPRESSION. **67.018**

SEMIGAMY

GENETIC CONTROL OF SEMIGAMY AND DERIVATION OF NULLISOMIC COTTON. 21.014, 21.015

GENETIC CONTROL OF SEMIGAMY; AND DERIVATION OF NULLISOMIC COTTON. 21.013

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003

SENSITIVITY

OEVELOPMENT OF A ONA HYBRIOIZATION ASSAY FOR OETECTION OF THE RING ROT PATHOGEN. 11.007 SEQUENCES

ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM

ALFALFA. 20.015

MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 SEQUENTIAL

RFLP METHOOOLOGY FOR RELATEONESS AMONG SMALL GRAINCEREAL ACCESSIONS. 18.002

SEROLOGICAL-TESTS

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

SEROLOGY

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

SHIITAKE

GERMPLASM ENHANCEMENT AND CULTURE OF EDIBLE MUSHROOMS. 12.038

SHOCK

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

SHOOTS

ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEDLING. 14.070

SHRUNKEN-2,

OISSOCIATION MUTAGENESIS OF THE SHRUNKEN-2 LOCUS OF MAIZE. 14.012

SIGNALS

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

SILVICULTURE

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

SLOW-RELEASE

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

SMALL-FRUITS

MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA OUCH.. 10.005

SMALL-GRAINS

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT STORAGE PROTEINS. 18.015

ANEUPLOIO ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*

PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047

TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

SOIL-BACTERIA

GLYPHOSATE OEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

SOIL-BORNE-DISEASES

ECOLOGY AND BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

SOIL-MICROORGANISMS

GLYPHOSATE OEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

SOIL-TEMPERATURE

ECOLOGY ANO BIOCONTROL OF SOILBORNE PATHOGENS. 13.003, 21.010*

SOLANACEAE

USE OF MOLECULAR MARKERS IN PLANT BREEDING AND GENETICS. 67.071

SOLANUM

ALTERNATIVE APPROACHES TO POTATO RING ROT RESEARCH AND VARIETAL IMPROVEMENT. 11.010 SYSTEMATICS OF POTATOES AND THEIR WILD RELATIVES (SOLANUM SECT. PETOTA). 11.017

SOLANUM,

EXPLORATORY RESEARCH ON THE CYTOGENETICS OF

SOLANACEOUS CROPS. 11.001

SOLAR-RADIATION

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. **67.046 SOLUBLE-PROTEINS**

BIOCHEMICAL AND DEVELOPMENTAL STUDIES OF PROTEINS ASSOCIATED WITH OXYGEN EVOLUTION. 67.087

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIOIZING POPULATIONS. 14.051

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 67.095

SOMATIC-CELLS

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*

ONA METHYLATION AND CONTROL OF GENE EXPRESSION IN SHOOT AND ROOT OF MAIZE SEEDLING. 14.070

INHERITANCE OF MITOCHONORIAL ONA IN SOMACLONAL VARIANTS. 20.003

SOMATIC-HYBRIDS

CONSTRUCTION AND CHARACTERIZATION OF SOMATIC HYBRIOS AND CYBRIOS OF TOMATO. 12.030

MITOCHONDRIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. **62.005**

SOMATIC-RECOMBINATION

EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032

SORGHUM

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*

COSMIO MAPPING OF MITOCHONORIAL ONA OF MALE FERTILE AND MALE STERILE SDRGHUM. **15.002**

CROP IMPROVEMENT AND GERMPLASM OEVELOPMENT THROUGH CHROMDSDMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

GENE REGULATION, EXPRESSION, AND MOLECULAR MAPPING IN MAIZE AND SDRGHUM. 14.007, 15.001*

GENETICS AND PHYSIDLOGY OF FUSARIA. 14.034, 15.004*, 17.018*

METHODS OF CONTROLLING CDRN INSECTS. 14.024
MOLECULAR MANIPULATION OF GENES. 28.002
ORGANELLE DNA DRGANIZATION AND CYTOPLASMIC

MALE STERILITY IN PENNISETUM. 20.005
RESTRICTION FRAGMENT LENGTH PDLYMORPHISMS IN SDRGHUM. 15.009

SOYBEAN

A PLANT MITOCHONORIAL MATURASE GENE. **67.099**MDLECULAR ANALYSIS OF A SDYBEAN TRANSPDSABLE ELEMENT. **23.023**

SOYBEAN-OIL

GENETIC ENGINEERING DF DILSEED SPECIES TD IMPRDVE DIL QUALITY. 23.020, 25.003*
REGULATION DF FATTY ACID SYNTHESIS PROTEINS IN SDYBEAN. 23.037

SOYBEANS

CELLULAR METABOLISM IN PLANTS. **67.049**CLASSICAL GENE MAPPING IN SOYBEAN USING MDLECULAR MARKERS. **23.014**

CLONING, EXPRESSION AND MUTAGENESIS DF SDYBEAN SEEDLING DIHYDROFOLATE REDUCTASE. 23.053

CONTROL OF THE BIOSYNTHESIS OF PROTEINS & AMIND AC IDS IN LEGUME SEEDS. 23.046

DEVELOPMENTAL REGULATION OF LIGHT-HARVESTING CDMPLEX GENES. 23.004

EFFICIENCY OF NITRDGEN FIXATION. 23.025,

EXPRESSION OF SOYBEAN NUCLEAR GENES IN

TRANSGENIC PLANTS. 23.007

GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033
GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS.
12.024, 16.005*, 23.032*, 63.003*

GENE MAPPING IN SOYBEAN WITH MOLECULAR MARKERS. 23.034, 23.056

GENETIC ENGINEERING OF RHIZOBIUM JAPONICUM, THE SOYBEAN SYMBIONT, FOR IMPROVED AGRONOMIC PROPERTIES. 23.039

GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*

GENETIC STRUCTURE OF CULTIVATEO SOYBEANS AND THE WILD SOYBEAN (GLYCINE SOJA) GERMPLASM. 23.045

GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

GLYPHOSATE OEGRADATION AND METABOLISM IN MICROORGANISMS. 23.026, 36.001*

ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*
MECHANISM DF ACTIDN OF PLANT GROWTH

MECHANISM DF ACTIDN OF PLANT GROWTH SUBSTANCES IN PLANT GROWTH AND DEVELOPMENT. **67.015**

MECHANISMS OF PATHOGENESIS AND RESISTANCE IN PLANT-PARASITE INTERACTIONS. 18.004, 23.005*

MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036
MODULATION AND NITROGEN FIXATION OF PRC R.
JAPONICUM. 23.021

MDLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDDMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022

MOLECULAR SYSTEMATICS OF SDYBEAN (GLYCINE: LEGUMINDSAE). 23.047

ORGANIZATION AND EXPRESSION OF LEGHEMDGLOBIN-LIKE SEQUENCES IN ALNUS GLUTINDSA. **67.092**

ORGANIZATION AND EXPRESSION OF SDYBEAN TRNA GENES. 23.055

PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH ONA METHYLATION. 23.028 REGULATION OF FATTY ACID SYNTHESIS PROTEINS

IN SOYBEAN. 23.037
REGULATION OF GENE EXPRESSION IN PLANTS.
14.020

REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003

REGULATION OF SDYBEAN SEEO PROTEIN GENE EXPRESSIDN. 23.001

RELATION OF ISOZYME LOCI TO QUALITATIVE CHARACTERS OF SDYBEAN. 23.017

SOYBEAN BROWN STEM ROT. 23.008

SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013

STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SDYBEAN INSECTION SEQUENCE. 23.035
STRUCTURE AND EXPRESSION OF SOYBEAN LEAF

STORAGE PROTEIN GENES. 23.043

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. 67.044

STRUCTURE AND REGULATION OF NIF GENES IN RHIZOBIUM FREOII, A NEWLY OESCRIBEO SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

SPATIAL-DISTRIBUTION

GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND DISEASES. 06.003

SPECIALTY-MUSHROOMS

GERMPLASM ENHANCEMENT AND CULTURE OF EOIBLE MUSHROOMS. 12.038

SPECIFICITY

CLONING CULTIVAR SPECIFICITY GENES FROM MAGNAPORTHE GRISEA. 16.010

OEVELOPMENT OF A ONA HYBRIOIZATION ASSAY FOR OETECTION OF THE RING ROT PATHOGEN. 11.007

SPECTROSCOPY

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. 67.046 SPINACH

THE THYLAKOID ENERGY TRANSOUCING ATPASE COMPLEX. 67.111

SPIROPLASMA

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

SPRAYS

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. 09.004

STABILITY

SYNTHESIS OF NOVEL GENOMIC COMBINATIONS IN MEDICAGO. 20.001

STARCH-DERIVATIVES

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

STARCH-SYNTHESIS

MUTANT GENES THAT AFFECT ENOOSPERM
OEVELOPMENT IN MAIZE. 14.078
REGULATION OF TUBER PROTEIN SYNTHESIS IN

STARCHES

MUTANT GENES THAT AFFECT ENOOSPERM OEVELOPMENT IN MAIZE. 14.078

STARTER-CULTURES

GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOOOS. 14.025, 31.001*

STATISTICAL-ANALYSIS

POTATO. 67.095

CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS. 14.032, 23.024*, 30.001*, 31.002*

STATISTICS

CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS. 14.032, 23.024*, 30.001*, 31.002*

STERILE-HYBRIDS

EXPERIMENTAL USE OF ISOZYMES IN APPLIEO PLANT GENETICS RESEARCH. 12.032

STERILITY

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003

STIMULATION

FUNCTION OF PLANT RNA-OEPENOENT RNA POLYMERASES. 12.036

STORAGE

MAIZE GRAIN PROTEIN COMPOSITION AND
OISTRIBUTION AS RELATED TO HARONESS,
HANDLING AND PROCESSING. 14.016
REGULATION OF FATTY ACIO SYNTHESIS PROTEINS
IN SOYBEAN. 23.037

STORAGE-PROTEINS

ANALYSIS OF THE DIFFERENTIAL SYNTHESIS OF OAT

STORAGE PROTEINS. 18.015

BIOCHEMISTRY OF GENETIC SYSTEMS. 14.033 CHARACTERIZATION OF GENES FOR STORAGE

PROTEINS OF WHEAT GRAIN. 17.012

EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*

GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005

GLIAOIN GENES OF COMMON WHEAT ANO ITS ANCESTORS. 17.011

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012

MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE OEVELOPMENT IN THE WHEAT PLANT. 17.003

MUTANT GENES THAT AFFECT ENOOSPERM OEVELOPMENT IN MAIZE. 14.078

ORGANIZATION ANO MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR REGULA- TION OF OAT STORAGE PROTEINS. 18.035

REGULATION OF GENE EXPRESSION IN PLANTS. 14.020

SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013

STRUCTURE AND EXPRESSION OF SOYBEAN LEAF STORAGE PROTEIN GENES. 23.043

WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

STORAGE-TISSUES

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 67.095

STRAIN-DIFFERENTIATION

RECOMBINANT ONA APPROACH TO DETECTION AND STRAIN DIFFERENTIATION OF THE BACTERIAL RING ROT PATHOGEN. 11.009

STRAINS

EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIDIZING POPULATIONS. 14.051
MOLECULAR GENETICS OF NITROGEN METABOLISM IN RHIZOBIA. 23.022

STRAINS-(GENETICS)

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*

THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045

STRAWBERRIES

MOLECULAR GENETIC STUDIES IN THE CULTIVATED STRAWBERRY, FRAGARIA X ANANASSA OUCH.. 10.005

STREET-TREES

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009

STRESS

CELLULAR ANO MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.027

STRESS-TOLERANCE

ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048

GENOME ORGANIZATION IN THE CULTIVATEO AND WILO SPECIES OF TOMATO. 12.015

GERMPLASM, GENETICS AND STRESS PHYSIOLOGY OF FOOD LEGUMES. 12.043, 62.007*

MOLECULAR GENETIC APPROACHES FOR GERMPLASM DEVELOPMENT OF FRUIT AND NUT CROPS. 10.001

STRUCTURAL-ANALYSIS

STRUCTURAL ANALYSIS, DISTRIBUTION, AND USES OF A SOYBEAN INSECTION SEQUENCE. 23.035

STRUCTURAL-GENES

A COMPARATIVE STUDY OF N(2)-FIXING GENES IN CLOSTRIDIUM PASTEURIANUM AND OTHER MICROORGANISMS. 67.103

BACTERIAL GENES COOING FOR PLANT RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE. **67.098** GENE STRUCTURE AND EXPRESSION IN PLANTS. **14.027**

MOLECULAR BIOLOGY OF THE PECTATE LYASE ISOZYMES OF ERWINIA CHRYSANTHEMI. 11.006 SOYBEAN STORAGE PROTEINS: BIOCHEMISTRY AND GENETICS. 23.013

STRUCTURAL-PROPERTIES

EXPRESSION OF MAIZE MITOCHONORIAL GENOME. 14.002

STRUCTURAL-PROTEINS

STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS IN MAIZE. 14.072

STRUCTURE

CELLULAR ANO MOLECULAR BIOLOGY OF SUGARCANE. 27.003

ORGANIZATION OF THE R CHROMOSOME REGION IN MAIZE. 14.077

SUBCELLULAR-BIOLOGY

GENETICS AND MOLECULAR BIOLOGY OF COTTON CYTOPLASMIC MALE STERILITY. 21.003

SUBERIN

BIOCHEMISTRY OF PLANT CUTICLE. **67.108** SUBSTITUTION

CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010 SUCROSE

MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011

SUCROSE-SYNTHASE

MOLECULAR CHARACTERIZATION OF THE SUCROSE SYNTHETASE-2 GENE OF MAIZE. 14.011

SUGARCANE

CELLULAR ANO MOLECULAR BIOLOGY OF SUGARCANE. 27.003

SUSCEPTIBILITY

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. **06.015**

GENETIC MOSAICS ANO THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND DISEASES. **06.003**

SUSPENSION-CULTURE

GENETIC REGULATION OF A SEEO-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

MITOCHONORIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. 67.030 SWEETPOTATOES

MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM OIVERSITY AND SYSTEMATICS. 12.020

SYMBIOSIS

AMINO ACIO METABOLISM ANO THE RHIZOBIUM-LEGUME SYMBIOSIS. **66.014**, **67.107***

ISOLATION ANO GENETICS OF GENES INVOLVED IN EXOPOLYSACCHARIOE BIOSYNTHESIS OF RHIZOBIUM JAPONICUM. 23.027

MAPPING THE SYMBIOSIS GENES OF PEA. 67.077
STRUCTURE AND REGULATION OF NIF GENES IN
RHIZOBIUM FREOII, A NEWLY OESCRIBEO
SPECIES OF SOYBEAN RHIZ. 23.051, 66.009*

SYSTEMATIC

SYSTEMATICS OF POTATOES AND THEIR WILO RELATIVES (SOLANUM SECT. PETOTA). 11.017

SYSTEMATICS

MOLECULAR MARKERS FOR EVALUATION OF SWEET POTATO GERMPLASM DIVERSITY AND SYSTEMATICS. 12.020

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE:

LEGUMINOSAE). 23.047
PRESERVATION AND UTILIZATION OF GERMPLASM IN COTTON. 21.016

TABTOXINS

MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUCOMONAOS. 12.046, 67.109*

TECHNIQUES

CELLULAR GENETICS OF CITRUS SPECIES. **09.002 TELOSOMES**

OEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002 PRESERVATION AND UTILIZATION OF GERMPLASM IN

COTTON. 21.001

TELOSOMICS

ANEUPLOIO ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007

GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC AND QUALITY CHARACTERISTICS OF OURUM AND COMMON WHEAT. 17.025

TEMPERATURE

ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

TEOSINTE

MEASURING THE GENETIC OIVERSITY OF CULTIVATEO PLANTS USING ISOZYME FREQUENCIES. 14.060 STRUCTURE, VARIATION AND INHERITANCE OF RIBOSOMAL GENE FAMILIES IN MAIZE. 14.036

TERPENES

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

THREE-DIMENSIONAL-MODELS

STRUCTURE AND MECHANISM OF ACTION OF SOYBEAN LIPOXYGENASE AND METABOLISM OF ITS PRODUCTS. 67.044

THREONINE

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

THYLAKOIDS

FUNCTIONAL AND MUTATIONAL ANALYSIS OF A THYLAKOIO PROTEIN. **67.020**THE THYLAKOIO ENERGY TRANSOUCING ATPASE COMPLEX. **67.111**

TIMBER

GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. **06.009**

TISSUE-CULTURE

BIOCHEMICAL AND DEVELOPMENTAL GENETICS OF HIGHER PLANTS. 14.038, 18.020

BIOLOGICAL CONTROL OF PLANT PATHOGENIC BACTERIA. 10.002, 12.018*

CELLULAR ANO MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*, 67.028

CELLULAR GENETICS OF CITRUS SPECIES. **09.002**CELLULAR METABOLISM IN PLANTS. **67.049**CHROMOSOMAL MAPPING OF GENES CONTROLLING
TISSUE CULTURE RESPONSE IN WHEAT. **17.010**CLASSICAL GENE MAPPING IN SOYBEAN USING

CLASSICAL GENE MAPPING IN SOYBEAN USING MOLECULAR MARKERS. 23.014

CONTROL OF GENE EXPRESSION IN POTATO. 11.002
CONTROL OF THE BIOSYNTHESIS OF PROTEINS &
AMINO AC IOS IN LEGUME SEEOS. 23.046

CROP IMPROVEMENT ANO GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL ANO CYTOPLASMIC MANIPULATIONS. 17.009

- DEVELOPMENT OF NEW AND IMPROVED CROPS FOR WATER CONSERVATION IN ARIO LANDS. 25.004
- DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS.
- EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032
- EXPRESSION OF EMBRYO-SPECIFIC GENES OURING IN VITRO EMBRYOGENESIS IN BRASSICA. 25.002, 67.042*
- GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033
 GENE MAPPING IN SOYBEAN WITH MOLECULAR
 MARKERS. 23.056
- GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 17.023*. 18.029*
- GENETICS AND CYTOLOGY OF MAIZE. 14.022
 GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016
- MITOCHONORIAL ONA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. 67.030 MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036
- MOLECULAR GENETIC CHANGES ASSOCIATED WITH SELECTION IN AGRONOMIC CROPS. 14.076, 20.016*
- PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011
 TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*
- TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*

TISSUE-CULTURE,

- TISSUE CULTURE GENETIC SYSTEMS. **67.059 TOBACCO**
 - CELLULAR METABOLISM IN PLANTS. **67.049**CHARACTERIZATION OF THE ROLE OF A STRESS
 PROTEIN (GENE(S) IN SALT WATER STRESS
 TOLERANCE IN PLANTS. **67.048**
 - DEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. 14.061
 - GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033
 - GENE EXPRESSION IN PLANTS: A STUDY OF POLYADENYLATION IN PLANTS. 20.007
 - GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 67.104
 - THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIDASE. 26.005
 - THE ROLE OF POLYAMINES DURING STRESS-INDUCED ALTERATIONS IN GENE EXPRESSION. 67.073
 - USE OF MOLECULAR MARKERS IN PLANT BREEDING AND GENETICS. 67.071

TOBACCO-VEIN-MOTTLING-VIRUS

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. 67.054

TOMATO

0

0

TAGGING PLANT GENES WITH TIGHTLY-LINKED RFLP MARKERS. 12.035

TOMATOES

- BIOLOGICAL CONTROL OF PLANT PATHOGENIC
- BACTERIA. 10.002, 12.018*
 CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.035
- CELLULASE GENE EXPRESSION DURING FRUIT DEVELOPMENT. 67.004
- CHILLING AND GENE EXPRESSION IN FRUIT. 12.006
 CONSTRUCTION AND CHARACTERIZATION OF SOMATIC
 HYBRIDS AND CYBRIOS OF TOMATO. 12.030
- EXPERIMENTAL USE OF ISOZYMES IN APPLIED PLANT GENETICS RESEARCH. 12.032
- FUNCTION OF PLANT RNA-DEPENDENT RNA POLYMERASES. 12.036
- FURTHER DEVELOPMENT OF PROTEIN-SPECIFIC GENETIC MARKERS IN TOMATO. 12.034
 GENOME ORGANIZATION IN THE CULTIVATED AND

- WILD SPECIES OF TOMATO. 12.015
- ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015
- ISOLATION OF THE RESISTANCE GENE IN TOMATO TO THE NEMATOOE MELOIOOGYNE INCOGNITA. **67.053**
- MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO. **65.001**, **67.012***
- MOLECULAR SWITCHES IN PLASTIO OIFFERENTIATION. 67.089
- REGULATION OF ETHYLENE INDUCED GENE EXPRESSION OURING FRUIT RIPENING. 67.009
- TOWAROS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017
- USE OF MOLECULAR MARKERS IN PLANT BREEDING AND GENETICS. 67.071
- VEGETABLE GENETICS. 12.014

TOXINS

- MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*
- PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011 SOYBEAN BROWN STEM ROT. 23.008

TRAITS

- CELLULAR AND MOLECULAR BIOLOGY OF SUGARCANE. 27.003
- DISSECTION OF HETEROSIS OF QUANTITATIVE TRAITS USING MOLECULAR MARKERS. 14.056
- ISOZYME LOCI AS MARKERS FOR LOCATING AND MANIPULATING QUANTITATIVE TRAIT LOCI.
 14.058
- MOLECULAR MARKERS IN MAIZE BREEDING AND GENETICS. 14.021
- THE MOLECULAR BIOLOGY OF THE RICE ALPHA-AMYLASE GENES. 16.001
- TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

TRANSCRIPTION

- A PLANT MITOCHONORIAL MATURASE GENE. **67.099**ANALYSIS OF THE OIFFERENTIAL SYNTHESIS OF DAT
 STORAGE PROTEINS. **18.015**
- CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*, 70.001*
- CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013
- CHARACTERIZATION OF THE MAIZE GENOME: SEQUENCES CODING FOR SPECIFIC GENES. 67.038
- CLONING OF SEQUENCES PROGRAMMING MEIOSIS IN NORMAL AND MUTANT ZEA MAYS. **67.014**
- EXPLOITATION OF RIBOSOMAL ONA TO CONTROL PLANT PATHOGENIC FUNGI. **67.101**
- EXPRESSION OF MAIZE MITOCHONORIAL GENOME. 14.002
- EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007
- GENETIC REGULATION OF A SEED-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*
- MITOCHONDRIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005
- MOLECULAR CONTROL OF GENE ACTIVITY DURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003
- PHOTOREGULATED GENE EXPRESSION IN CHLOROPLASTS. 67.082
- REGULATION OF GENE EXPRESSION IN PLANTS. 14.020
- REGULATION OF PLANT GENE EXPRESSION BY ONA METHYLATION. 67.110
- REVERSE TRANSCRIPTASE AND REPLICATION OF

CAULIFLOWER MOSAIC VIRUS. 67.061 STRUCTURE AND EXPRESSION OF PLANT GENES ENCOOING CALMOOULIN. 18.014 STRUCTURE, EXPRESSION AND EVOLUTION OF

CYANOBACTERIAL GENES REGULATED OURING NITROGEN FIXATION. 67.084

THE PHYCOCYANIN GENES OF AGMENELLUM QUADRUPLICATUM. 67.088

TUBULIN GENES OF PLANTS. 67.060

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTEO CELLS. 67.054

TRANSCRIPTIONAL-REGULATION

ANALYSIS OF SEEO GLOBULIN GENES AND THEIR EXPRESSION IN CEREALS. 18.016

ISOLATION AND CHARACTERIZATION OF GENES REGULATED BY LIGHT. 67.080

MECHANISMS DIRECTING HORMONAL AND OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026

PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081

REGULATION OF GENE EXPRESSION IN PLANTS. 14.020

THE PHYTOCHROME GENE: STRUCTURE, ORGANIZATION AND EXPRESSION. 18.036, 63.007*
THE REPLICATION OF CHLOROPLAST ONA IN

CHLAMYOOMONAS REINHAROII. 67.057

TRANSDUCERS

THE THYLAKOIO ENERGY TRANSOUCING ATPASE COMPLEX. 67.111

TRANSDUCTION

THE THYLAKOIO ENERGY TRANSOUCING ATPASE COMPLEX. 67.111

TRANSFERASES

MOLECULAR CLONING OF THE RUBBER TRANSFERASE GENE FROM GUAYULE. 28.004

PLANT PROTEINS INVOLVED IN REGULATING GENE EXPRESSION THROUGH DNA METHYLATION. 23.028 REGULATION OF PLANT GENE EXPRESSION BY DNA

METHYLATION. **67.110**THE GLUTATHIONE S-TRANSFERASE GENES FROM CORN ANO COTTON. 14.068, 21.007*

TRANSFORMATION

BASIC AND APPLIED MOLECULAR GENETICS OF THE POTATO BACTERIAL RING ROT PATHOGEN. 11.008 CONTROL OF GENE EXPRESSION IN POTATO. 11.002 OEVELOPMENT OF NEW AND IMPROVED CROPS FOR WATER CONSERVATION IN ARIO LANOS. 25.004

OEVELOPMENT OF NONSEXUAL TECHNIQUES FOR GENETIC ENGINEERING OF ZEA MAYS L.. 14.061

OEVELOPMENTAL REGULATION OF LIGHT-HARVESTING COMPLEX GENES. 23.004

EXPRESSION OF SOYBEAN NUCLEAR GENES IN TRANSGENIC PLANTS. 23.007

GENETIC ENGINEERING OF OILSEED SPECIES TO IMPROVE OIL QUALITY. 23.020, 25.003*

ISOLATING AND CHARACTERIZING A-BETA MATING-TYPE ALLELES OF SCHIZOPHYLLUM COMMUNE.. 66.013, 67.102*

ISOLATION AND TRANSFORMATION OF A WOUND-INDUCED TRYPSIN INHIBITOR GENE FROM ALFALFA. 20.015

ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055

THE PHYCOCYANIN GENES OF AGMENELLUM QUAORUPLICATUM. 67.088

TOWARDS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

TRANSGENIC

THE MOLECULAR CHARACTERIZATION OF THE LIGNIN-FORMING PEROXIOASE. 26.005

TRANSGENIC-PLANTS

CONTROL OF GENE EXPRESSION IN POTATO. 11.002 EXPRESSION OF MELANIN IN PLANTS - MONITORING

GENE EXPRESSION. 12.002, 13.001, 67.001 REGULATION OF PLANT GENE EXPRESSION. 67.106 TRANSLATION

CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013 TRANSLATION-(GENETICS)

MITOCHONORIAL GENOME FUNCTION IN CYTOPLASMIC MALE STERILE AND FERTILE PLANTS. 62.005

TRANSLATIONAL-CONTROL

COOING AND REGULATION OF MITOCHONORIAL GENES. 14.052

TRANSLOCATION

GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016

PHYSIOLOGY OF PARASITISM AND CONTROL OF PHYMATOTRICHUM OMNIVORUM. 21.011

STUDIES OF CHROMOSOME TRANSLOCATIONS IN MAIZE & THEIR USE AS RESEARCH TOOLS. 14.018

TRANSLOCATION-(GENETICS)

CORN IMPROVEMENT THROUGH RESEARCH IN GENETICS AND CYTOGENETICS. 14.043
CYTOGENETIC ANALYSIS OF RESTRICTION FRAGMENT

LENGTH POLYMORPHISMS (RFLPS). 14.015

DEVELOPMENT OF A CYTOGENETIC MANIPULATION SYSTEM FOR COTTON. 21.002

OEVELOPMENT OF A GENETIC MANIPULATION SYSTEM FOR COTTON. 67.024

GENETIC CONTROL OF PLANT MORPHOGENESIS: STUDIES ON TWO HOMEOTIC MUTANTS OF MAIZE. 14.065

GENETICS AND CYTOGENETICS OF SOYBEANS. 23.015 **TRANSPORT**

OEVELOPMENTAL REGULATION OF SPECIFIC HEAT SHOCK PROTEINS. 12.003, 63.001*

TRANSPOSABLE

MOLECULAR ANALYSIS OF A SOYBEAN TRANSPOSABLE ELEMENT. 23.023

TRANSPOSABLE-ELEMENTS

OISSOCIATION MUTAGENESIS OF THE SHRUNKEN-2 LOCUS OF MAIZE. 14.012

GENE EXPRESSION IN PLANT DEVELOPMENT. 23.033 MOBILE ELEMENTS IN THE SOYBEAN GENOME. 23.036 TISSUE CULTURE GENETIC SYSTEMS. 14.041, 18.022*

TRANSPOSABLE ELEMENTS IN MUTATOR ACTIVITY IN MAIZE. 14.026

TRANSPOSABLE-ELEMENTS.

GENETIC/MOLECULAR CHARACTERIZATION OF THE MUTATOR-RELATEO CY TRANSPOSABLE ELEMENT SYSTEM OF MAIZE. 14.031

TRANSPOSITION

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*

REPEATEO DNA SEQUENCES AND CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008

TRANSPOSITION,

GENETIC/MOLECULAR CHARACTERIZATION OF THE MUTATOR-RELATED CY TRANSPOSABLE ELEMENT SYSTEM OF MAIZE. 14.031

TRANSPOSON

THE MOLECULAR BASIS OF BLACK ROT OF CRUCIFERS. 12.001

TRANSPOSON,

TRANSPOSON MUTAGENESIS IN TOMATO. 12.016 TRANSPOSONS

ACTIVATION OF THE MU FAMILY OF TRANSPOSABLE ELEMENTS. 14.003

CELLULAR AND MOLECULAR GENETICS FOR CROP

IMPROVEMENT. 18.013, 26.002*
OEVELOPMENTAL GENETICS USING THE ALCHOL OEHYOROGENASE GENE-SYSTEM IN MAIZE. 14.004

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 12.010

- GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004
- HOST-PATHOGEN RECOGNITION AND DISEASE RESISTANCE IN PLANTS. 67.113
- ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE FUNCTION AND REGULATION. 14.054
- ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIDATE GENE STRUCTURE, REGULATION AND FUNCTION. 14.059
- ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*
- ISOLATION OF TRANSPOSON INDUCED MAIZE MUTANTS OFFECTIVE IN THE BIOSYNTHESIS OF ABSCISIC ACIO. 14.001
- MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUDOMONAOS. 12.046, 67.109*
- PLASMIOS IN PLANT PATHOGENIC BACTERIA. 18.012, 26.001*
- TOWAROS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

TREE-AGE

GENETIC MOSAICS ANO THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND DISEASES. **06.003**

TREE-BREEDING

- OEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS.

 O6.008
- GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014
- GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. **06.009**
- PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEEO ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. **06.013**

TREE-DISEASES

- GENETIC MOSAICS ANO THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND DISEASES. **06.003**
- PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO OISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

TREE-FORMS

.

- GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014
- GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

TREE-GENETICS

- CHARACTERIZATION OF THE NATURAL VARIABILITY IN PINUS RADIATA ONA. 06.011
- CYTOGENETIC STUDIES OF HARDWOOD AND CONIFEROUS FOREST TREES. **06.016**
- DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS. 06.008
- GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014
- GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. 06.009
- INHERITANCE AND PHENOTYPIC SIGNIFICANCE OF CHLOROPLAST DNA POLYMORPHISMS IN LODGEPOLE AND JACK PINES. **06.004**
- PATTERNS OF GENETIC VARIATION AMONG BREEDING/SEED ZONES OF OOUGLAS-FIR IN SOUTHERN OREGON. 06.013

TREE-GROWTH

- GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014
- GENETIC MOSAICS AND THEIR RELATIONSHIP TO PATTERNS OF SUSCEPTIBILITY TO INSECTS AND

- OISEASES. 06.003
- GENETICS AND IMPROVEMENT OF NORTHEASTERN TREES. **06.009**

TREE-IMPROVEMENT

- OEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS.

 06.008
- GENETIC IMPROVEMENT OF NORTHWEST TREES. 06.014

TREE-PHYSIOLOGY

GENETIC STRUCTURE AND ADAPTATION OF NATURAL POPULATIONS OF SPRUCE. **06.007**

TREES

CYTOGENETIC STUDIES OF HAROWOOD AND CONIFEROUS FOREST TREES. **06.016**GENETIC IMPROVEMENT OF NORTHWEST TREES.

06.014 TRI-ACYLGLYCEROLS

STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS IN MAIZE. 14.072

TRIACYLGLYCEROL

GENETIC ENGINEERING OF OILSEED SPECIES TO IMPROVE OIL QUALITY. 23.020, 25.003*

TRIFOLIUM-SUBTERRANEUM

REPEATEO ONA SEQUENCES ANO CHLOROPLAST ONA INSTABILITY IN CLOVER. 20.008

TRISOMICS

- ANEUPLOIO ANALYSIS OF GENETIC ARCHITECTURE OF BARLEY CHROMOSOMES. 18.007
- BARLEY GENETICS AND PLANT CYTOGENETICS.
- 18.008, 20.004*, 27.001*, 67.022*

 GENETIC AND LINKAGE STUDIES IN BARLEY. 18.006

 GENETICS AND CYTOGENETICS OF SOYBEANS, 23.015
- GENETICS OF SOYBEAN WITH SPECIAL EMPHASIS ON GENE MAPPING. 23.016
- ORGANIZATION OF ALPHA AMYLASE GENES IN BARLEY. 18.017

TRITICEAE

- CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*
- GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOIO WHEAT AND ITS RELATIVES. 67.097

TRITICUM

- CHROMOSOMAL LOCALIZATION AND INTRACHROMOSOMAL MAPPING OF WHEAT RFLP LOCI. 17.032
- CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIOIZE TO UNIQUE-SEQUENCE-ONA CLONES. 17.031
- EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACID SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*
- GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS IN HEXAPLOID WHEAT ANO ITS RELATIVES. 67.097

TROPICAL-FRUIT

PATHO-PHYSIOLOGY OF POST-HARVEST DISEASES OF TROPICAL FRUIT AND THEIR CONTROL. **09.004**

TRYPSIN

REGULATION OF SOYBEAN PROTEIN GENE EXPRESSION. 23.003

TSUGA-HETEROPHYLLA

GENETIC IMPROVEMENT OF NORTHWEST TREES.

06.014

TUBERIZATION

CONTROL OF GENE EXPRESSION IN POTATO. 11.002
ISOLATION AND CHARACTERIZATION OF POTATO
TUBERIZATION GENES. 11.003

TUBERS

CONTROL OF GENE EXPRESSION IN POTATO. 11.002 CYTOPLASMIC FACTORS OF THE POTATO. 11.012 OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015 REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO: 11.014, 67.094*, 67.095

TUBULIN

TUBULIN GENES OF PLANTS. 67.060

TYROSINE

BIOCHEMISTRY OF PLANT CUTICLE. 67.108

ULTRAVIOLET-RADIATION

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 63.002, 66.002*, 67.026*, 70.001*

LIREASE

GENETIC REGULATION OF A SEEO-SPECIFIC PROTEIN IN SOYBEAN. 23.041, 62.003*, 63.004*

GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67.063*

UREIDES

GENETIC REGULATION OF THE SOYBEAN UREASE ISOENZYMES. 23.042, 62.004*, 63.005*, 67 063*

VAIRIMORPHA-NECATRIX

METHOOS OF CONTROLLING CORN INSECTS. 14.024 **VARIANTS**

GENETICS AND PHYSIOLOGY OF FUSARIA. 14.034, 15.004*, 17.018*

MITOCHONORIAL DNA VARIATION IN SUBPOPULATIONS OF SOMATIC HYBRIO CELLS OF GRASSES. 67.030

VARIEGATION

HYBRID VARIEGATION IN PHASEOLUS VULGARIS. 23.038

VARIETIES

ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013

VARIETY-TESTS

ISOLATION AND CHARACTERIZATION OF CORN GENES TO ELUCIOATE GENE FUNCTION AND REGULATION. 14.054

PLANT GERMPLASM INTRODUCTION, INCREASE, EVALUATION, OOCUMENTATION, MAINTENANCE, ANO DISTRIBUTION. 25.001

VECTOR-HOST-RELATIONS

MECHANISMS DIRECTING HORMONAL AND OEVELOPMENTAL REGULATION OF GENE EXPRESSION IN BARLEY. 18.026

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 67.027

CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010

CLONING, CHARACTERIZING AND TRANSFERRING GENES IN RICE. 16.006

OEFINING ANO MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*

OEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS. 06.008

ISOLATION OF GENES IMPLICATED IN THE CONTROL OF IMPORTANT AGRONOMIC CHARACTERS. 14.017, 23.010*

MOLECULAR MANIPULATION OF GENES. 28.002 ORGANIZATION AND EXPRESSION OF SOYBEAN TRNA GENES. 23.055

TOWARDS A TRANSPOSON TAGGING SYSTEM IN TOMATO. 12.017

VEGETABLE-OILS

STRUCTURE OF GENES INVOLVEO IN OIL SYNTHESIS IN MAIZE. 14.072

VEGETABLE-PROTEINS

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. **67.095**

VEGETABLES

GENE MAPPING IN ECONOMICALLY IMPORTANT CROPS. 12.024, 16.005*, 23.032*, 63.003* GENETIC ENGINEERING TO IMPROVE PLANT HEALTH

AND PRODUCTION EFFICIENCY. 06.018, 12.010, 12.048*. 14.079*

GENETICS AND BREEDING OF COOL SEASON CROPS. 12.011

GENOME EVOLUTION IN BRASSICA. 12.013 ISOLATION AND CHARACTERIZATION OF POTATO TUBERIZATION GENES. 11.003

REGULATION OF TUBER PROTEIN SYNTHESIS IN POTATO. 67.095

RELATIONSHIPS AMONG LIGHT, PHOTOSYNTHETIC CO(2) ASSIMILATION, AND RUBISCO ACTIVITY IN PLANTS. 12.023

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006* VEGETABLE GENETICS. 12.014

VEGETATIVE-COMPATIBILITY

RESEARCH ON THE INTRASPECIFIC VARIATION IN CENOCOCCUM GEOPHILUM FR., 67.076

VESICULAR

EVALUATION AND ENHANCEMENT OF WHEAT AND DAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

VIROLOGY

THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045

VIRULENCE

GENETICS OF BIOTYPES IN THE HESSIAN FLY

(MAYETIOLA OESTRUCTOR). 17.014 HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM: BACTERIAL BLIGHT OF RICE. 16.002

HOST-PATHOGEN RECOGNITION AND DISEASE

RESISTANCE IN PLANTS. 67.113
MOLECULAR AND GENETIC BASIS OF PATHOGENESIS OF PSEUDOMONAS SPECIES. 67.034

MOLECULAR BASIS OF RACE-SPECIFIC AVIRULENCE IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. 23.002, 66.001*

MOLECULAR GENETICS OF PATHOGENICITY AND VIRULENCE IN PHYTOPATHOGENIC PSEUOOMONAOS. 12.046, 67.109*

VARIATION IN LETTUCE OOWNY MILDEW. 12.008 VIRUS-ASSAYS

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

VIRUS-CELL-INTERACTIONS

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTEO CELLS. 67.054

VIRUS-CHARACTERIZATION

BIOLOGY, EPIDEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045

VIRUS-DETECTION

BIOLOGY, EPIDEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

VIRUS-DISEASES-(INSECTS)

IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIOES BY RECOMBINANT ONA TECHNOLOGY. 65.002, 67.031*

VIRUS-DISEASES-(PLANTS)

BIOLOGY, EPIOEMIOLOGY & CONTROL OF VIRUSES & MYCOPLASMA-LIKE ORGANISMS CAUSING DISEASES OF CORN. 14.035, 15.005*

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

DEFINING ANO MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*

OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING OISEASE RESISTANCE INTO POTATOES. 11.018

GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004

PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047

THE NATURE OF RESISTANCE TO PLANT VIRUSES. 12.045

VIRUS-DNA

OEFINING ANO MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*

ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. **65.004**, **67.040***

THE NATURE OF RESISTANCE TO PLANT VIRUSES.
12.045

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTEO CELLS. 67.054

VIRUS-GENETICS

BIOLOGY, EPIOEMIOLOGY, GENETICS, AND CONTROL OF VIRUSES AND MOLLICUTES CAUSING DISEASES OF CORN. 14.066, 66.010*

OEFINING ANO MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*

IMPROVING THE EFFICACY OF BACULOVIRUS
 PESTICIOES BY RECOMBINANT ONA TECHNOLOGY.
65.002, 67.031*

ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. **65.004, 67.040***

THE NATURE OF RESISTANCE TO PLANT VIRUSES.
12.045

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. 67.054

VIRUS-REPLICATION

PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047

THE NATURE OF RESISTANCE TO PLANT VIRUSES.
12.045

VIRUS-RNA

ORGANIZATION AND EXPRESSION OF A BACULOVIRUS ONA GENOME. **65.004, 67.040***

VIRAL GENE EXPRESSION IN POTYVIRUS INFECTED CELLS. **67.054**

VIRUS-STRUCTURE

PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047

VIRUS-TRANSMISSION

PATHOGENESIS AND RELATIONSHIPS OF PLANT VIRUSES. 67.047

VIRUS-X-(POTATOES)

GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004

VIRUSES

CELLULAR AND MOLECULAR GENETICS FOR CROP IMPROVEMENT. 14.009, 15.003*, 23.006*, 24.001*

OEFINING AND MAPPING THE GENES OF CAULIMOVIRUSES. 66.004, 67.055*

OEVELOPMENT OF MOLECULAR BIOLOGY MEANS FOR INCOR- PORATING OISEASE RESISTANCE INTO POTATOES. 11.018

ENTOMOPATHOGENS FOR USE IN PEST MANAGEMENT SYSTEMS. **06.015**

GENETIC IMPROVEMENT OF POTATO: INCORPORATION OF EXTREME RESISTANCE TO POTATO VIRUS X. 11.004

REVERSE TRANSCRIPTASE AND REPLICATION OF CAULIFLOWER MOSAIC VIRUS. 67.061

VITAMIN-A

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

VITAMIN-E

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

VITIS

GENETICS, PHYSIOLOGY AND MOLECULAR BIOLOGY OF HOST-PATHOGEN INTERACTION. 10.008, 66.012*

VVVEGETABLES

BREEDING, DISEASES AND CULTURE OF DRY PEAS AND LENTILS. 12.040, 62.006*

WALL

CELL WALLS OF MAIZE PERICARP. 15.010

WATER-STRESS

CHARACTERIZATION OF THE ROLE OF A STRESS PROTEIN (GENE(S) IN SALT WATER STRESS TOLERANCE IN PLANTS. 67.048

WATERSHED-MANAGEMENT

CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHOOS. 17.007

WATERSHEDS

CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA METHODS. 17.007

WAVELENGTH

PHOTOREGULATEO GENE EXPRESSION IN CHLOROPLASTS. 67.081

WEED-CONTROL

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 17.023*, 18.029*

WESTERN-BLOTTING

FUNCTION OF PLANT RNA-OEPENOENT RNA POLYMERASES. 12.036

WHEAT

CELLULAR REGULATION OF THE EXPRESSION OF OORMANT GENES IN CEREAL GRAINS. **62.002**CEREAL GRAIN IMPROVEMENT BY RECOMBINANT ONA

METHOOS. 17.007

CHARACTERIZATION OF GENES FOR STORAGE PROTEINS OF WHEAT GRAIN. 17.012

CHROMOSOMAL LOCALIZATION AND INTRACHROMOSOMAL MAPPING OF WHEAT RFLP LOCI. 17.032

CHROMOSOMAL LOCATIONS AND EVOLUTION OF HOMOLOGOUS UNIQUE SEQUENCE GENES IN TRITICEAE SPECIES. 17.030, 18.032*

CHROMOSOMAL LOCATIONS OF WHEAT GENES THAT HYBRIOIZE TO UNIQUE-SEQUENCE-ONA CLONES. 17.031

CHROMOSOMAL MAPPING OF GENES CONTROLLING TISSUE CULTURE RESPONSE IN WHEAT. 17.010

CROP IMPROVEMENT AND GERMPLASM DEVELOPMENT THROUGH CHROMOSOMAL AND CYTOPLASMIC MANIPULATIONS. 17.009

EVALUATION AND ENHANCEMENT OF WHEAT AND OAT ACCESSIONS IN THE NATIONA L SMALL GRAINS COLLECTIO. 17.008, 18.011*

EVOLUTION OF POLYPLOIO WHEATS VIA AMINO ACIO SEQUENCING AND ELECTROPHORETIC STUDIES. 17.004, 18.005*

GENE LOCATIONS FOR WHEAT ECONOMIC TRAITS BY RECIPROCAL CHROMOSOME SUBSTITUTIONS.

17.024

GENE SYNTENY RELATIONSHIPS AND MAP LOCATIONS

IN HEXAPLOIO WHEAT AND ITS RELATIVES. 67.097

GENETIC AND CYTOGENETIC ANALYSIS OF AGRONOMIC ANO QUALITY CHARACTERISTICS OF OURUM ANO COMMON WHEAT. **17.025**

GENETIC ASPECTS AND BIOSYNTHESIS OF WHEAT PROTEINS. 17.005

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. 07.001, 17.023*, 18.029*

GLIAOIN GENES OF COMMON WHEAT AND ITS ANCESTORS. 17.011

ISOLATIONS, MOLECULAR CHARACTERIZATIONS & CLONING OF GENES FROM MUTANTS REGULATING LYSINE IN CEREALS. 16.003, 17.019*, 23.030*, 26.003*

MAPPING, TRANSFER, RECOMBINATION AND EXPRESSION OF ORGANELLE AND NUCLEAR GENES. 16.004, 17.020*, 23.031*, 67.056*

MOLECULAR CONTROL OF GENE ACTIVITY OURING REPRODUC TIVE DEVELOPMENT IN THE WHEAT PLANT. 17.003

ORGANIZATION AND MANIPULATION OF WHEAT STORAGE PROTEIN GENES. 17.013

PHYSICAL MAPPING OF THE GENOME OF WHEAT. 17.016

RECOMBINANT ONA APPROACHES TO PLANT GENE STRUCTURE AND GENOME DIVERSITY. 14.064, 17.026*, 18.030*, 67.085*

TISSUE CULTURE AND THE TRANSFER OF CYTOPLASMIC GENES. 17.027, 18.031*

TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

WHEAT STORAGE PROTEIN GENE CONTROL SYSTEMS. 17.006, 67.021*

WILD-DATS

18.003, 67.006*

WILD-PLANTS

GENETICS, TISSUE CULTURE, AND MOLECULAR BIOLOGY OF THE SOYBEAN. 23.018

MOLECULAR GENETIC EVALUATION OF PLANT GERMPLASM RESOURCES. 67.017

MOLECULAR SYSTEMATICS OF SOYBEAN (GLYCINE: LEGUMINOSAE). 23.047

TRANSFER OF QUANTITATIVE TRAITS: ELYTRIGIA SALT TOLERANCE TO WHEAT BY CHROMOSOME ENGINEERING. 17.001

WOODY-PLANTS

OEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR GENETIC IMPROVEMENT OF WOODY PLANTS. 06.008

WOUNDS

OIFFERENTIAL EXPRESSION OF PROTEINASE INHIBITOR GENES IN PLANT TISSUES. 11.015

ISOLATION AND TRANSFORMATION OF A
WOUND-INDUCEO TRYPSIN INHIBITOR GENE FROM
ALFALFA. 20.015

STRUCTURE, EVOLUTION AND FUNCTION OF PLANT PROTEINASE INHIBITORS. 11.016, 12.044*, 20.014*, 26.006*, 63.006*

W4

MOLECULAR ANALYSIS OF A SOYBEAN TRANSPOSABLE ELEMENT. 23.023

X-RAY-DIFFRACTION

STRUCTURE OF PHOTOSYNTHETIC MEMBRANES. **67.046 XANTHOMONAS**

GENETIC ENGINEERING TO IMPROVE PLANT HEALTH AND PRODUCTION EFFICIENCY. **07.001**, **17.023***, **18.029***

THE MOLECULAR BASIS OF BLACK ROT OF

CRUCIFERS. 12.001

XANTHOMONAS-CAMPESTRIS

HOST-PATHOGEN INTERACTIONS IN A MODEL SYSTEM:
BACTERIAL BLIGHT OF RICE. 16.002

PATHOGENSIS, MOLECULAR BIOLOGY AND BIOLOGICAL CONTROL OF XANTHOMONAO DISEASES OF FRUIT TREES. 09.006, 10.004*, 66.005*

XENOBIOTICS

PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. **67.041**

YEASTS

CHARACTERIZATION OF THE MAIZE GENOME: CLONING OF SPECIFIC MAIZE GENES INTO YEAST. 14.013 GENE STRUCTURE AND EXPRESSION IN PLANTS. 14.027

ISOLATION OF GENES THAT ENCODE DNA BINDING PROTEINS. **67.066**

MANIPULATION OF STORAGE PROTEIN STRUCTURE IN SOYBEANS. 23.012

YIELD-RESPONSE

STRUCTURE OF GENES INVOLVED IN OIL SYNTHESIS IN MAIZE. 14.072

YUGOSLAVIA

ISOZYME VARIATION AMONG MAIZE COLLECTIONS FROM YUGOSLAVIA ANO OTHER EUROPEAN COUNTRIES. 14.080

ZEA-MAYS,

GENETIC/MOLECULAR CHARACTERIZATION OF THE MUTATOR-RELATED CY TRANSPOSABLE ELEMENT SYSTEM OF MAIZE. 14.031

ZEIN

BIOCHEMICAL CHARACTERIZATION OF STORAGE PROTEINS, LIPIOS, AND NATURAL ANTIOXIDANTS IN CORN. 14.014

MOLECULAR MAPPING OF GENES IN CORN. 14.040

2.4-D

PLANT CYTOCHROMES P-450 REGULATION OF GENE EXPRESSION. **67.041**

2N-GAMETES,

EXPLORATORY RESEARCH ON THE CYTOGENETICS OF SOLANACEOUS CROPS. 11.001

ABBOTT A G. 10.009, 13.002, CASHMORE A R. 67.074 23.054* CHANDRA G R. 62.001, 62.002 ADAMS W T. 06.013 ALLARD R W IN THE GENUS AVENA. 18.003, 67.006* AMASINO R M. 67.110 **AN G**. 67.106 ANDERSON 0 D. 17.005, 17.006, 17.007, 67.021* ATHERLY A G. 23.021 **AXELROD B.** 67.044

1

1

BACKHAUS R A. 28.004 BALLARD R E. 13.002 BECKETT J B. 14.043, 14.044, 14.046 BENNETT A B. 12.009 BERNATZKY R. 12.026 BERRYHILL D L. 14.064, 17.026*, 18.030*, 67.085* **BILLARD R**. 14.016 **BILLARD R**. 10.009, 23.054* 23.050, 66.006*, 66.007*, 66.008* BLACK W C. 17.014 BLAKE T K. 07.001, 17.023*, CULLIS C A. 06.011 18.027, 18.028, 18.029* CURTIS S E. 67.084 BOCKELMAN H E. 17.008, BOE A A. 11.010, 11.011 BOGORAD L. 67.058 BOHNERT H J. 12.002, 13.001, 67.001 BOSTON R S. 67.078, 67.079 DELLAPORTA S L. 14.053 BOYER C D. 67.089 BRAY E A. 67.015, 67.016 BRAYMER H D. 23.026, 36.001* BRESSAN R. 67.048 BRONSON C R. 67.051 BROWSE J A. 25.006, 28.005*, 62.008* BULLA L. 06.018, 12.048*, 14.079* BURGESS D. 14.006 BUSHNELL W R. 17.021

CALDWELL K A. 17.004, 18.005* CAMPBELL D E. 17.006, 67.021* CARMAN J G. 67.100 CASHMORE A. 67.091

CHANG H. 06.010 CHATTERJEE A K. 67.052 CHELM B K. 23.039 CHEN J S. 67.103 CHERRY J H. 67.049
CHIN C. 10.005
CHOUREY P S. 14.007,
14.009, 15.001*, 15.003*,

FISCHER R L. 12.006, 14.009, 15.001*, 15.003*, 23.006*, 24.001* 12.009, 67.008, 67.009

CHRISTOFFERSEN R E. 67.004

CIVEROLO E L. 09.006, FOLK W R. 23.055

FOX T D. 14.052

TO 004* 66.005* FOX T D. 14.052 10.004*, 66.005*

CLEGG M T. 67.017

COE E H. 14.043, 14.044,

FREELING M. 14.004 COLBERT J T. 18.009 COLE R. 11.012 COLLMER A. 11.006 COMPTON W A. 14.049 CONDIT C M. 67.065 COOKSEY D A. 10.002, 12.018*, 12.019 23.032*, 63.003* CROUCH M C. 25.002, 67.042* GAYDA R C. 23.027 GENDEL S M. 23.022 CULLEN D. 06.017

DANDEKAR A M. 10.001 DAVIS L C. 23.025, 66.003* DEAN D R. 23.057, 23.058 DENNY T P. 67.034, 67.035 DEVINE T E. 23.029 **DEZOETEN G A.** 11.018 **DOEBLEY J F.** 15.006 DOYLE G G. 14.044, 14.046 DOYLE J J. 20.011, 23.047 DURBIN R D. 16.010 DUYSEN M E. 17.027, 18.031* **DVORAK J.** 17.001, 17.002

ECKERT R T. 06.009 EHRLICH K C. 23.028 EHRLICH M. 23.028 ELKAN G H. 23.051, 66.009* ELLINGBOE A H. 66.015, 67.112* ENDO R M. 18.004, 23.005* GURLEY W B. 63.002, ENDRIZZI V. 21.001 ERBACH D C. 14.024

EVENSEN K B. 67.089

FEHR W R. 23.017 FERGUSON N H. 14.071, FREISHEIM J H. 23.053 FRIESEN P D. 65.003, 6/.039*
FRITZ P J. 10.007
FRYXELL P A **FRYXELL P A.** 21.016 FULLINGTON J G. 17.004, 18.005*

> GALAU G A. 21.003 GALINAT W C. 14.037 GENGENBACH B G. 14.038, 14.042, 18.020 GERIC I. 14.080 GILL B S. 17.010, 17.015, 17.016, 17.017 **GINGERY R E**. 14.066, 66.010* GLATZ B A. 14.025, 31.001* GOLD M H. 06.012, 66.011* **GOLDBERG R B**. 23.001, 23.003 GOODMAN M M. 14.060 GOTH R W. 11.009 **GOTTLIEB L D**. 67.007 GRAY L E. 23.008 GREEN D E. 23.019 GREENE F C. 17.003, 17.005, 17.006, 17.007, 67.021* GREENWOOD M. 06.006 GROVE G. 14.068, 14.069, 21.007* GRUMET R. 12.027 GRUN P. 11.012 GUDMESTAD N C. 11.007, 11.008, 11.009, 11.010, 11.011 GUILFOYLE T J. 67.061 66.002*, 67.025, 67.026*, 70.001*

GUSTAFSON J P. 18.024 **GUTHRIE W D**. 14.024 **GUY C L**. 67.027

HAMMOND E G. 23.020, 25.003* HANG A. 18.008, 20.004*, 27.001*, 67.022*

HANNAH L C. 14.011, 14.012

HANNEMAN R E. 11.018

Z1.010* HANOVER J W. 06.007

HANSON A D. 18.019

HANSON, M R. 62.005, 67.072

HARDISON R C. 67.089

HARRISON R G. 14.051

KINLAW C S. 06.002 HART G E. 15.009, 17.030, KLEIN A. 14.050
17.031, 17.032, 18.032*, KLEINHOFS A. 18.034, 17.013 HEINZ D J. 27.003 KEINING KNAPP S J. 25.004 KNIGHT R J. 27.002 KOFOIO K D. 14.064, HEINZ D J. 27.003 HELENTJARIS T. 14.075
HELENTJARIS T G. 14.015
HELENTJARIS T G. 14.015
HELGESON J P. 11.018

HELGESON J P. 11.018 HELGESON J P. 11.018 HEPBURN A G. 14.017, 23.010* HERRMANN K M. 67.045 KRIZ A L. 14.019 MUTSCHLER M A. 12.033, 67.070 HIEBERT E. 14.009, 15.003*, KRUL W R. 10.008, 66.012* MYERS A M. 14.029, 14.030 23.006*, 24.001*
HILU K W. 24.002
HOCKETT E A. 18.028
HOFFMAN D L. 17.008, 18.011* HOLLAND M. 10.005 HOOD E E. 15.010 HOUSTON D B. 06.003 HOUSTON D R. 06.003 HOUTZ R L. 12.023 HUANG A. 14.072 HUANG A H C. 14.005

INGRAM L O. 63.002, 66.002*, 67.026*, 70.001*

HUNT A G. 20.007 HUTCHISON K. 06.006 HUTCHISON K W. 06.005

JACKSON A 0. 67.047 JAIN S K. 25.001 JARRET R L. 09.003, 12.020, 67.029 JELENKOVIC G. 10.005 JOHNSON C. 09.003 MA D P. 15.007 JOHNSON J. 06.018, 12.048*, MADISON J T. 23.046 14.079* JOHNSON J L. 67.103 JOHNSON J S. 15.008, 17.028*, 21.009*, 35.001*, 40.001* JONES R J. 14.042 JOPPA L R. 17.025

KAHLER A L. 25.005, 28.003* MCOONALD B A. 17.029, KAHN M L. 66.014, 67.107* KAO T H. 11.013, 67.090* KASARDA D D. 17.003. 17.004, 17.005, 18.005*

KEATHLEY D E. 06.007,
06.008 KOLATTUKUDY P E. 67.108 KORBAN S S. 09.005, 10.003*

LAGRIMINI M. 26.005 LAMKEY K R. 14.021 NASKALEAN 0 B. 67.067,

LAMKEY K R. 14.021 NEALE 0 B. 06.001, 06.002

LARK G A. 23.056 NELSON 0 E. 14.078

LARKINS B A. 14.020, 18.016 NEUFFER M G. 14.047

LARSON G E. 25.005, 28.003* NIBLETT C L. 14.007, LARUE T A. 67.077 LEACH J E. 16.002 LEE M. 14.021, 14.029, 14.030 **LEONG S**. 16.010, 67.113 LESLIE J F. 14.034, 15.004*, 17.018* LEVINGS C S. 14.061 **LEWIS S A.** 10.009, 23.054* LI N. 14.068, 21.007* LIANG G H. 17.009 LITTS J C. 17.003 LOMMEL S. 14.035, 15.005* LOUIE R. 14.066, 66.010* LYOA S O. 21.011

MAGILL C. 14.074, 16.008*
MALLET J L B. 21.004 MATHEWS. 12.024, 16.005*, 23.032*, 63.003*

MATHEWS B F. 23.034

MATTHEWS B F. 12.025, 16.004, 17.020*, 23.031*, PALUKAITIS P F. 12.036 67.056* MAXWELL D. 16.010 MAXWELL D. 10.010
MCCARTHY W J. 06.015
MCCORMICK S. 12.004
MCCOY T J. 20.001, 20.002
MCCOY T J. 20.001, 20.002
PATTERSON E B. 14.018
PAULIS J W. 14.016

20.013* MCKNIGHT T D. 21.012 MCMULLEN M. 14.066, 66.010* MEAGHER R. 23.007 MEAUMER R B. 67.032
MENZEL M Y. 21.002, 67.024
MENZEL M Y. 21.002, 67.024
MGAILL C. 15.009
MICHELMORE R W. 12.010,
67.013
KENERLEY C M 10.000 MEAGHER R B. 67.032 MICLELMORE R W. 12.008 MILES 0. 14.048 MILLER L K. 65.002, 65.004, 67.031*, 67.040* MIRCETICH S M. 18.002 MIRCEITCH S M. 18.002 MOGEN B D. 11.007 MOORE G A. 09.002 MOORE P H. 27.003 MORRIS O W. 14.026, 14.027 MORRIS M R. 17.024 MUEHLBAUER F J. 12.039, 12.040, 12.041, 12.042, 12.043, 62.006*, 62.007* MUTHUKRISHNAN S. 18.017, 18.018, 62.001

> **NASRALLAH J B.** 67.067. 15.001* NIELSEN N C. 23.012, 23.013 NIKOLAU B J. 23.020, 25.003*

> O'CONNELL M A. 12.030. 12.031, 21.006* **OHLROGGE J B**. 23.037 OISHI K K. 14.001 OKITA T W. 16.009, 17.033* **OKUBU C**. 12.020 **OLESON A E**. 11.007, 11.008, 11.009, 11.010, 11.011, 14.064, 17.026*, 18.030*, 67.085* **OSBORN T C.** 14.076, 20.016* **0ZIAS-AKINS P J.** 67.030

> PALMER J D. 20.008 PALMER R G. 23.014, 23.015, 23.016, 23.017, 23.018, 23.021, 23.023, 23.056 PAPA K E. 67.036 PARK W D. 11.014, 67.094*, PATIL S S. 09.004, 12.021

 PETERSON D M.
 18.035
 SCHNABLE P S.
 14.031,

 PETERSON P A.
 14.022,
 23.023
 PHILLIPS R L. 14.039, POLACCO J C. 23.041, POLACCO J C. 23.041, 23.042, 62.003*, 62.004*, 63.005*, 67.063*
POLLAK E. 14.032, 23.024*, 30.001*, 31.002*
POLLAK L. 14.021
POPE D D. 67.037
PRIBNOW D G. 06.012, 66.011*
PRICE H J. 21.012
PRING D R. 14.009, 15.002, 15.002, 15.003*, 20.005, 23.006*, 24.001*
POLACK E. 23.041, 06.001*
SEDEROFF R R. 06.001, 21.001
SELMAN B R. 67.111
SEQUEIRA L. 11.019, 67.113
SEQUEIRA L. 11.019, 67.113
TYE B K. 67.066
TULEEN N A. 17.032
TYE B K. 67.066
TYE B K. 67.066
TULEICH R C. 66.013
FAMW J J. 12.001
SHAW J J. 12.001
SHAW P D. 18.012, 18.013, 67.101, 67.102*
ULLRICH R C. 66.013
67.101, 67.102*
UPCHURCH R G. 23.05
SHEPHERD R J. 66.004, 67.055*
SHERIDAN W F. 14.065 24.001*

RUSSELL W A. 14.024 RYAN C A. 11.015, 11.016, 12.044*, 20.014*, 20.015, 26.006*, 63.006*

.

.

4)

1

SALIN M L. 67.062 SANDS D C. 07.001, 17.023*, SANDS D C. 07.001, 17.023*, 23.009

TEW T L. 27.003

SCANDALIOS J G. 14.062

THEOLOGIS A. 09.001, YAMAGUCHI J. 12.004

YENDOL W G. 06.015

YODER J I. 12.015, 12.016

16.003, 16.005*, 17.019*, 23.030*, 23.032*, 26.003*, THOMAS M D. 13.003, 21.010*

THOMAS M D. 13.003, 21.010*

THOMAS M D. 13.003, 21.010*

YOUNG N D. 12.035

THOMPSON J F 23.046

SHERIDAN W F. 14.065 SHERIDAN W F. 14.065
SHIBLES R M. 23.019
SHOEMAKER R. 23.014
SHOEMAKER R. 23.014
VASIL I K. 67.028
VASIL V. 67.028
VIERLING E. 12.003,
67.010
SILEN R R. 06.014
63.001*, 67.002
VODKIN L O. 23.011, 23.035,
67.010
OUALSET C O. 18.002
SINCLAIR - H. 67.042 QIN M. 60.00...

QUAIL P H. 18.036, 63.00.

67.010

QUALSET C O. 18.002

QUIROS C F. 11.001, 12.011,

12.012, 12.013

SINS U U.

SINCLAIR J H. 67.043

SINK K. 12.028

SINK K C. 12.029

SISCO P H. 14.054, 14.059

WAGNER D B. 06.004

PLIGAR S G. 67.041

WALBOT V. 14.002,

14.047

WALBOT V. 14.002,

14.047

WALBURG G. 18.015 SLIGAR S G. 67.041 WALBOT V. 14.002, 14.003,

> TABITA F R. 67.098 TANKSLEY S D. 12.032, 12.034, 12.035, 67.071 TAVANTZIS S. 11.004, 11.005 TBD. 09.001, 12.005*,

PETERSON D M. 18.035 SCHNABLE P S. 14.031, 10BIN E M. 67.005, 67.020 TOWN C D. 67.086 TSUCHIYA T. 18.006, 18.007, 14.041, 18.021*, 18.022*, 67.059 POLACCO J C. 23.041, 23.042, 62.003*, 62.004*

ULLRICH R C. 66.013, UPCHURCH R G. 23.052

RAMAGE R T. 18.001
REECK G R. 14.033
REED S M. 26.004
REEDS R N. 25.005, 28.003**
RHODES R R. 23.033
RICK C M. 12.014
ROBERTS D F. 67.023
ROBERTS D D S. 14.028, 67.070
ROBERTS D W. 66.015, 67.102**
ROBERTS D W. 66.015, 67.102**
ROBERTS D W. 66.015, 67.102**
ROBERTS D W. 66.015, 67.014
ROBERTS D G I 8.025
ROBERTS D G I 8.025
ROBERTS D G I 8.025
ROBERT S D G I 8.026
ROBERT S D G I 8.025
ROBERT S D G I 8.026
ROBERT S D G I 8.025
ROBERT S D G I 8.026
ROBERT S D G I 8.026
ROBERT S D G I 8.025
ROBERT S D G I 8.026
ROBERT S D G I 8.026 WU R. 16.006, 16.007 WU Y V. 14.016 WUNDER B A. 18.009 WURTELE E S. 23.020, 25.003* WYATT S D. 12.045

ZEMETRA R S. 17.024 ZIELINSKI R E. 18.014 ZIMMER E A. 14.036

ALABAMA

Auburn University, AUBURN 12.001

ARIZONA

Agricultural Research Service, TUCSON 18.001 University of Arizona, TUCSON 12.002, 12.003, 13.001, 14.001, 20.001, 20.002, 20.003, 21.001, 63.001*, 67.001, 67.002

CALIFORNIA

Agricultural Research Service, ALBANY 09.001, 12.004, 12.005*
Western Regional Res Center, ALBANY 17.003, 17.004, 17.005, 17.006, 18.005*, 67.021*
800 Buchanan Street, ALBANY 17.007

University of California, BERKELEY 12.006, 14.004, 23.002, 66.001*, 67.008, 67.009, 67.010

1960 Addison Street, BERKELEY 06.001.

1960 Addison Street, BERKELEY 06.001, 06.002

University of California, DAVIS 10.001, 11.001, 12.007, 12.008, 12.009, 12.010, 12.011, 12.012, 12.013, 12.014, 12.015, 12.016, 12.017, 16.001, 17.001, 17.002, 18.003, 25.001, 65.001, 67.006*, 67.007, 67.011*, 67.012*, 67.013

Hunt Hall-university of Cal, DAVIS 18.002

University of California-san Diego, LA JOLLA 67.014

University of California, LOS ANGELES 23.001, 23.003, 67.005
University of Los Angeles, LOS ANGELES 67.020

6701 San Pablo Ave., OAKLAND 14.006

University of California, RIVERSIDE 10.002, 12.018*, 12.019, 14.005, 18.004, 23.004, 23.005*, 67.015, 67.016, 67.017, 67.018, 67.019

Salk Inst For Biological Studies, SAN DIEGO 67.003

University of California, SANTA BARBARA 67.004

Stanford University, STANFORD 14.002, 14.003

COLORADO

Colorado State University, FORT COLLINS 18.006, 18.007, 18.008, 18.009, 20.004*, 27.001*, 67.022*, 67.023

CONNECTICUT

51 Mill Pond Road, HAMDEN 06.003

FLORIDA

University of Florida, GAINESVILLE 09.002, 14.007, 14.008, 14.009, 14.010, 14.011, 14.012, 15.001*, 15.002, 15.003*, 18.010, 20.005, 20.006, 23.006*, 24.001*, 28.001, 28.002, 63.002, 66.002*, 67.025, 67.026*, 67.027, 67.028, 70.001*

Florida International University, MIAMI 12.020

Florida State University, TALLAHASSEE 21.002, 67.024

GEORGIA

University of Georgia, ATHENS 09.003, 21.003, 23.007, 27.002, 65.002, 67.031*, 67.032, 67.033, 67.034, 67.035, 67.036, 67.037

Agricultural Research Service, EXPERIMENT 67.029

Georgia Coastal Plain Expt Sta, TIFTON 67.030

HAWAII

Hspa, Po Box 1057, AIEA 27.003

University of Hawaii, HONOLULU 09.004, 12.021, 14.013, 67.038

•)

6

I DAHO

Agricultural Research Service, ABERDEEN 17.008, 18.011*

University of Idaho, MOSCOW 65.003, 65.004, 67.039*, 67.040*

ILLINOIS

809 South Wright Street, CHAMPAIGN 14.019, 18.014, 67.041

Illinois State University, NORMAL 14.015

Northern Regional Res Center, PEORIA 14.016

Agricultural Research Service, URBANA 14.014, 23.008, 23.009 1301 West Gregory Drive, URBANA 09.005, 10.003*, 14.017, 14.018, 18.012, 18.013, 23.010*, 23.011, 26.001*, 26.002*

INDIANA

Box 1847, BLOOMINGTON 25.002, 67.042* Indiana University, BLOOMINGTON 67.043

Agricultural Research Service, WEST LAFAYETTE 23.012
Purdue Res Foundation, WEST LAFAYETTE 18.015
Purdue University, WEST LAFAYETTE 14.020, 18.016, 23.013, 67.044, 67.045, 67.046, 67.047, 67.048, 67.049

IOWA

Agricultural Research Service, AMES 23.014, 23.015, 23.016

Iowa State University, AMES 14.021, 14.022, 14.023, 14.024, 14.025, 14.026, 14.027, 14.028, 14.029, 14.030, 14.031, 14.032, 23.017, 23.018, 23.019, 23.020, 23.021, 23.022, 23.023, 23.024*, 25.003*, 30.001*, 31.001*, 31.002*, 67.050, 67.051

KANSAS

Kansas State University, MANHATTAN 12.022, 14.033, 14.034, 14.035, 15.004*, 15.005*, 16.002, 17.009, 17.010, 17.011, 17.012, 17.013, 17.014, 17.015, 17.016, 17.017, 17.018*, 18.017, 18.018, 23.025, 62.001, 66.003*, 67.052, 67.053

KENTUCKY

University of Kentucky, LEXINGTON 06.004, 12.023, 20.007, 66.004, 67.054, 67.055*

LOUISIANA

Louisiana State University, BATON ROUGE 14.036, 23.026, 23.027, 36.001*

Po Box 19687, NEW ORLEANS 23.028

MAINE

University of Maine, ORONO 06.005, 06.006, 11.002, 11.003, 11.004, 11.005

MARYLAND

Beltsville Agr Res Center, BELTSVILLE 09.006, 10.004*, 12.024, 16.003, 16.004, 16.005*, 17.019*, 17.020*, 23.029, 23.030*, 23.031*, 23.032*, 23.033, 23.034, 26.003*, 62.002, 63.003*, 66.005*, 67.056*
Rm 101 Bldg 008, BELTSVILLE 12.025
Seed Research Laboratory, BELTSVILLE 23.035

()

University of Maryland, CATONSVILLE 23.036 University of Md 5401 Wilkens Ave, CATONSVILLE 67.057

University of Maryland, COLLEGE PARK 11.006

MASSACHUSETTS

University of Massachusetts, AMHERST 12.026

Harvard University, CAMBRIDGE 67.058

University of Massachusetts, WALTHAM 14.037

MICHIGAN

University of Michigan, ANN ARBOR 20.008

Michigan State University, EAST LANSING 06.007, 06.008, 12.027, 12.028, 12.029, 18.019, 23.037, 23.038, 23.039

MINNESOTA

Agricultural Research Service, ST PAUL 17.021 University of Minnesota, ST PAUL 14.038, 14.039, 14.040, 14.041, 15.006, 18.020, 18.021*, 18.022*, 67.059, 67.060, 67.061 1919 University Avenue, ST PAUL 14.042

MISSISSIPPI

Mississippi State University, MISSISSIPPI STATE 15.007, 21.004, 67.062

MISSOURI

Agricultural Research Service, COLUMBIA 14.043, 14.044, 14.045, 17.022*, 18.023*, 20.009*, 21.005*, 23.040*
University of Missouri, COLUMBIA 14.046, 14.047, 14.048, 18.024, 23.041, 23.042, 62.003*, 62.004*, 63.004*, 63.005*, 67.063*, 67.064

Washington University, ST LOUIS 18.025, 18.026

MONT ANA

Montana State University, BOZEMAN 07.001, 17.023*, 18.027, 18.028, 18.029*

NEBRASKA

University of Nebraska, LINCOLN 14.049, 17.024, 23.043

NEVADA

University of Nevada, RENO 20.010, 67.065

NEW HAMPSHIRE

University of New Hampshire, DURHAM 06.009, 14.050, 23.044, 23.045

NEW JERSEY

4

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Rutgers University Po Box 231, NEW BRUNSWICK 10.005

NEW MEXICO

New Mexico State University, LAS CRUCES 12.030, 12.031, 12.032, 21.006*

NEW YORK

P 0 Box 100, COLO SPRING HARBOR 14.053, 67.073

N Y Agriculture Expt Station, GENEVA 10.006, 12.037*

Agricultural Research Service, ITHACA 23.046
Cornell University, ITHACA 12.033, 12.034,

12.035, 12.036, 14.051, 14.052, 16.006, 16.007, 20.011, 23.047, 62.005, 67.066, 67.067, 67.068, 67.069, 67.070, 67.071, 67.072

Tower Road, ITHACA 67.077

Rockefeller University, NEW YORK 67.074, 67.075

State University of New York, SYRACUSE 67.076

NORTH CAROLINA

Agricultural Research Service, RALEIGH 14.054, 14.055, 14.056, 14.057, 14.058 North Carolina State University, RALEIGH 06.010, 14.059, 14.060, 14.061, 14.062, 14.063, 23.048, 23.049, 23.050, 23.051, 23.052, 26.004, 66.006*, 66.007*, 66.008*, 66.009*, 67.078, 67.079, 67.080, 67.081, 67.082, 67.083, 67.084

NORTH DAKOTA

Agricultural Research Service, FARGO 17.025
North Oakota State University, FARGO 11.007, 11.008, 11.009, 11.010, 11.011, 14.064, 17.026*, 17.027, 18.030*, 18.031*, 67.085*

University of North Oakota, GRANO FORKS 14.065

OHIO

Western Reserve University, CLEVELAND 06.011, 67.086

Medical College of Ohio, TOLEOO 23.053

Agricultural Research Service, WOOSTER 14.066, 66.010*
Ohio State University, WOOSTER 26.005

OREGON

Oregon Graduate Center, BEAVERTON 06.012, 66.011*

Oregon State University, CORVALLIS 06.013, 25.004, 67.087
Pacific NW Forest & Rge Exp Sta, CORVALLIS 06.014

PENNSYLVANIA

University of Pennsylvania, PHILAOELPHIA 67.091

Pennsylvania State University, UNIVERSITY PARK 06.015, 10.007, 11.012, 11.013, 12.038, 14.067, 14.068, 14.069, 21.007*,

67.088, 67.089, 67.090*

RHODE ISLAND

University of Rhode Island, KINGSTON 10.008, 14.070, 66.012*

SOUTH CAROLINA

Clemson University, CLEMSON 10.009, 13.002, 14.071, 20.012*, 23.054*

University of South Carolina, COLUMBIA 14.072

SOUTH DAKOTA

S Dakota State University, BROOKINGS
25.005, 28.003*

TENNESSEE

University of Tennessee, KNOXVILLE 06.016, 67.092

TEXAS

University of Texas, AUSTIN 23.055, 67.098

Agricultural Research Service, COLLEGE STATION 21.008
Pb Box 3578, COLLEGE STATION 67.093
Texas A&M University, COLLEGE STATION 11.014, 13.003, 14.073, 14.074, 15.008, 15.009, 16.008*, 17.028*, 17.029, 17.030, 17.031, 17.032, 18.032*, 20.013*, 21.009*, 21.010*, 21.011, 21.012, 21.013, 21.014, 21.015, 21.016, 35.001*, 40.001*, 67.094*, 67.095, 67.096, 67.097

Po Box 388, WESLACO 28.004

UTAH

Utah State University, LOGAN 15.010, 67.100

Native Plant Institute, SALT LAKE CITY 14.075 University of Utah, SALT LAKE CITY 23.056, 67.099 417 Wakara Way, SALT LAKE CITY 18.033

VERMONT

University of Vermont, BURLINGTON 66.013, 67.101, 67.102*

VIRGINIA

Virginia Poly Inst, BLACKSBURG 23.057, 23.058, 24.002, 67.103

1

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() ...

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WASHINGTON

Agricultural Research Service, PULLMAN 12.039, 12.040, 12.041, 12.042, 12.043, 62.006*, 62.007*
Washington State University, PULLMAN 11.015, 11.016, 12.044*, 12.045, 16.009, 17.033*, 18.034, 20.014*, 20.015, 25.006, 26.006*, 28.005*, 62.008*, 63.006*, 66.014, 67.104, 67.105, 67.106, 67.107*, 67.108

WISCONSIN

Agricultural Research Service, MADISON 11.017, 12.046, 18.035, 67.109*
One Gifford PinchOt Drive, MADISON 06.017
University of Wisconsin, MADISON 11.018, 11.019, 12.047, 14.076, 14.077, 14.078, 16.010, 18.036, 20.016*, 63.007*, 66.015, 67.110, 67.111, 67.112*, 67.113

WYOMING

University of Wyoming, LARAMIE 06.018, 12.048*, 14.079*

YUGOSLAVIA

Inst of Field & Veg Crops, NOVI SAD 14.080

